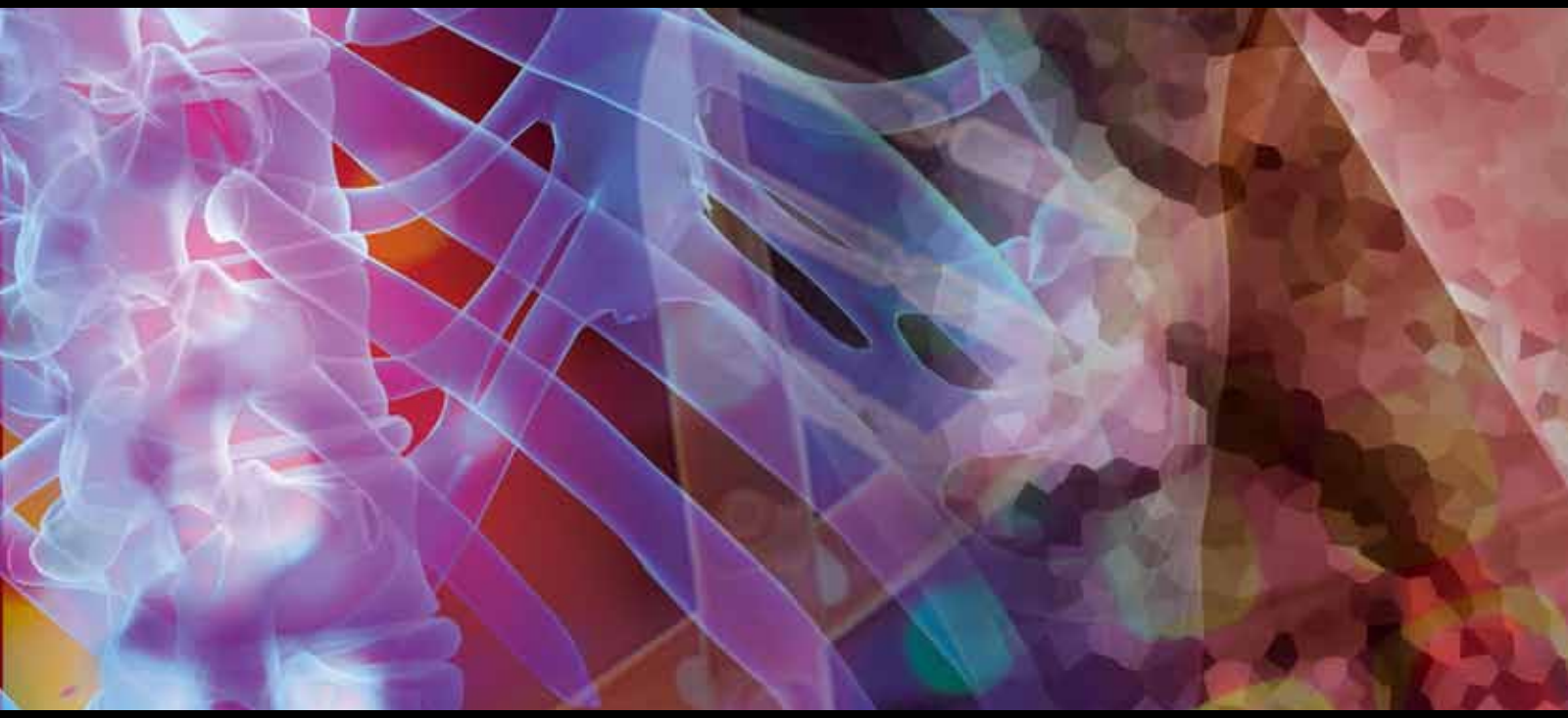
Current Gerontology and Geriatrics Research

Mechanisms and Implications of Age-Related Changes in the Liver

Guest Editors: Victoria C. Cogger, Sarah N. Hilmer, and Dmitri Svistounov



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Editorial Board

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Editorial

Mechanisms and Implications of Age-Related Changes in the Liver

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As the world's population ages in unprecedented numbers and proportions, management of the basic health needs of an older population is a key challenge [1]. Older age is unquestionably associated with increased vulnerability and susceptibility to disease and disability [2]. Ageing is the major independent risk factor for most diseases of the Western world including atherosclerosis, cancer, and arthritis as well as for the prototypical aging diseases such as dementia and osteoporosis [3]. Despite this, our understanding of how old age predisposes us to disease remains rudimentary [4].

A greater understanding of the ageing process will provide insight into the underlying causes of many diseases and open up new avenues for prevention and therapy. Our research into the underlying causes of ageing led us to examine the liver architecture in great detail. The age-related decrease in liver function is substantial and very relevant for systemic exposure to substrates implicated in disease pathogenesis and ageing. In the past, the liver was considered to be relatively unaffected by ageing and age-related diseases. Functional changes, particularly related to impaired drug metabolism (which is reduced by 40–50% in old age), were attributed only to age-related reduction in blood flow and liver mass [5]. However, as such mechanisms are unable to fully explain age-related impairment of hepatic function and the systemic effects of these changes, much greater investigation into other hepatic factors such as liver blood vessel ultrastructure, immune function, and gene and protein expression is warranted.

This special issue brings together many of the current areas of research in the liver and ageing. It details findings on the systemic role of the liver in health, disease, and treatment options in the setting of old age.

The first paper in this issue addresses the very important area of DNA repair in ageing in the liver. M. Lebel et al. have written an eloquent review that thoroughly explores the role of genetic instability in age-related loss of liver function. The authors postulate that this is an area of great promise in understanding and combating diminished liver function with age.

S. J. Mitchell et al. question the evidence base for the current prescribing guidelines for the common analgesic paracetamol in older people in their manuscript, which examines how poorly understood pharmacodynamics and pharmacokinetics are in the older person. Better understanding is required to reduce the risks both of underdosing this important analgesic and of causing accidental hepatotoxicity.

D. L. Schmucker and H. Sanchez investigate the impaired liver regeneration seen in older people and animal models and conclude that the regenerative capacity of older liver is not impaired, rather the rate of regeneration is reduced. This conclusion has very important implications for the contentious issue of the use of donor livers from older people in liver transplantation.

A. Warren et al. present an ultrastructural study of the liver in old age, with particular focus on the hepatic stellate cell, a cell synonymous with fibrotic liver changes in disease. The study shows for the first time that, while there is lipid engorgement of the cells with ageing, there is no activation. Smooth muscle actin expression, the hallmark of the hepatic stellate cell dedifferentiation into a fibroblast, is seen in many chronic liver diseases but not in old age. This finding yet again shows that ageing changes in the liver are distinct from the pathological processes seen in disease states.

X. He et al. address the important issue of cancer therapy in the older person and how liver-related changes drastically affect the efficacy and toxicity of chemotherapy agents. Due to comorbidities and poor functional status, older people have generally been omitted from drug therapy trials in this area. The authors argue that the key to increasing treatment efficacy and decreasing toxicity in this group is tailoring treatment specifically to individuals with their age and comorbidities foremost in their treatment plan.

Finally the paper by L. Gan et al. examines nonalcoholic fatty liver disease (NAFLD) in ageing. NAFLD is the most common liver disease today and most people diagnosed with NAFLD are aged over 60 years. The authors point out that "Advanced age is associated with disease severity and fibrosis progression; a relatively high proportion of individuals with progressive forms of NAFLD develop cirrhosis by the time they are in their 70s or beyond, although more data are required on the exact risks." The paper also presents some management strategies for older people with NAFLD.

These articles present the state of knowledge of many aspects of the liver in ageing. We would suggest that they put forward a compelling argument that a thorough understanding of how the liver changes with age is important in disease prevention, modulation, and treatment in the setting of the older person.

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Review Article

Mechanisms and Implications of Age-Related Changes in the Liver: Nonalcoholic Fatty Liver Disease in the Elderly

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Nonalcoholic fatty liver disease (NAFLD) is hepatic steatosis associated with metabolic abnormalities such as overweight/central obesity, insulin resistance, type 2 diabetes (T2D), and dyslipidemia. NAFLD is becoming the most common liver disease in contemporary society, with the highest prevalence in those over 60 years. NAFLD pathology ranges from simple steatosis to a necroinflammatory fibrosing disorder called steatohepatitis (SH), the latter associated with high risk of developing cirrhosis, often occuring in the seventh to ninth decades of life. While the main health implications of NAFLD are increased risk of developing T2D, cardiovascular diseases, and common cancers, there is substantantially increased standardized mortality, and deaths from decompensated cirrhosis and hepatocellular carcinoma (HCC). Little is known about the interactive effects of ageing and NAFLD, with most studies focusing on the younger population. This paper summarises the epidemiology, pathogenesis, and clinical course of NAFLD, with particular attention to persons over age 60 years. An approach to the management of NASH and its complications in the elderly, will also be presented here.

1. Introduction

In 1980, Ludwig and colleagues introduced the concept of nonalcoholic steatohepatitis (NASH) to describe liver histologic changes resembling alcoholic hepatitis in individuals without significant alcohol intake [1]. NASH is now conceptualized as part of a pathological spectrum of a fatty liver disorders caused by metabolic factors and referred to collectively as nonalcoholic fatty liver disease (NAFLD). The mildest form of NAFLD is simple steatosis, characterised by hepatic fat (triglyceride, TG) accumulation alone. In NASH, hepatic necroinflammatory changes are also present and a characteristic perisinusoidal pattern of liver fibrosis is common. Up to 25% of NAFLD patients have NASH, and in perhaps one third of such cases there is slowly progressive liver fibrosis leading to cirrhosis. Primary liver cancer (hepatocellular carcinoma, HCC) is now a recognized complication of NAFLD, usually but not always after development of cirrhosis [2–5]

Ludwig's descriptions of NASH suggested higher prevalence in women, particularly in those who are obese and have type 2 diabetes (T2D) [1]. Over the last decade, community-based studies have found male predominance of NAFLD from the paediatric population [6] up to fifth decade of life in adults. After age 60 years, however, females overtake their male counterparts in prevalence of NAFLD [7], an age and gender distribution that resembles that of cardiovascular disease. This is not surprising given similar risk factors for NAFLD and cardiovascular diseases.

Most cases of NAFLD occur in overweight or obese individuals, and there are particular strong links to central obesity, T2D, atherogenic dyslipidemia, and hypertension, each of which are elements of metabolic syndrome [8, 9]. NAFLD can now be regarded as the hepatic manifestation of the metabolic syndrome, although it is not yet included as a definitional component. The links between NAFLD and metabolic syndrome/prediabetes/T2D seem likely to reflect the operation of shared pathogenic factors, as we and others have reviewed [10–12]. Thus, the presence of fatty liver is a strong, independent predictor for the future development of metabolic syndrome [13–15], T2D, and cardiovascular events. A minority of cases of fatty liver (not due to alcohol)

are secondary to specific etiologic agents such as drugs or occur in well-defined settings (jejuno-ileal bypass, total parenteral nutrition); they are not regarded as NAFLD (which infers a metabolic etiology) and will be not discussed further here [12, 16, 17].

In this paper, we will first review the epidemiology and pathophysiology of NAFLD, with particular focus on age-related aspects. We will then examine risk factors for developing progressive liver injury and how known age-related changes in liver biology might contribute to severity of the disease among older people. The paper will conclude with some comments about management strategies that may be applicable to older individuals with fatty liver.

2. Epidemiology

2.1. Diagnosis. The current diagnosis of NAFLD is based on detection of hepatic steatosis by liver biopsy or imaging, exclusion of other liver diseases, particularly alcohol and hepatitis C, and recognition of metabolic risk factors. The true prevalence of NASH, the most clinically relevant subset of patients with NAFLD, has been difficult to establish because this is a histologic diagnosis. The diagnosis of NAFLD often comes to light because of detection of abnormal liver tests, particularly raised serum alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) and serum ferritin.

All noninvasive methods for diagnosis of NAFLD have limitations—computerised tomography (CT) scan and hepatic ultrasonography (US) are relatively insensitive; they can only detect moderate to severe steatosis [5, 18]. Serum ALT increase is not only insensitive, but also nonspecific; values may remain within the normal range in up to 80% of patients with biopsy-proven NAFLD and up to 30% of NASH [19]. Similarly, serum ferritin is a nonspecific inflammatory marker, but while it is not specific for diagnosis of NAFLD, once the diagnosis has been established, serum ferritin has been shown to be significantly more elevated in patients with NASH compared with those with simple steatosis (SS) [20].

The "gold standard" of NASH diagnosis is liver biopsy. However, apart from being an unpleasant and occasionally dangerous test, it is subject to sampling error and observer differences in histopathologic interpretation. Further, reported studies of NASH prevalence may suffer from selection and ascertainment biases as liver biopsies were often performed in specific groups like the morbidly obese and diabetics or among highly selected subjects enrolled in clinical trials [21].

In the last decade, transient elastography (TE) has been developed as a safe noninvasive alternative to liver biopsy for assessing liver fibrosis. This technique has been validated across different liver diseases, including NAFLD [22]. A recent study of 246 biopsy-proven NAFLD patients from Hong Kong and France suggested decreasing ability to successfully obtain liver stiffness measurements (LSM) with increasing BMI; measurement was 97% successful with BMI $< 30\,kg/m^2$, dropping to only 75% in obese patients, though this did not reach statistical significance [23]. However, the recent introduction of the "obese" probe allows LSM to be successfully increased from 45% to 76% in the morbidly obese (mean BMI $> 40\,kg/m^2$) [24]. In the elderly population, TE is a promising noninvasive method of quantifying liver fibrosis in NAFLD.

2.2. Community Prevalence of NAFLD and NASH: Rising Global Prevalence. Depending on the screening tool and when the study was performed, estimated community prevalence of NAFLD has ranged from 2.8% to 46% [25–27]. In the Dallas Heart Study, Browning et al. used proton magnetic resonance spectroscopy (MRS) to quantify hepatic triglyceride (TG) content of 3 major ethnic groups. Hepatic steatosis (defined as greater than 5.5% TG content) was noted in 31% overall, with significant ethnic variation—45% in hispanics, 33% in whites, and 24% in blacks [28]. In Japan, Korea, Taiwan, and China, the use of ultrasonography in community studies has found rates between 5 and 40% [29], with an increasing prevalence over the last 20 years [29, 30]. A recent study in predominantly middle-aged American employees or outpatients (without known liver disease) found that the prevalence of steatosis by ultrasonography was 47%; in this study, NASH was confirmed histologically in 12% of the total cohort or 30% of the ultrasound-positive subgroup [27]. Another recent analysis of nearly 40,000 patients from three cycles of the National Health and Nutritional Examination Survey (NHANES) conducted between 1988 and 2008 showed rising prevalence of NAFLD from approximately 5% in the 1988–1994 cohort to 11% in the 2005–2008 cohort; similarly, they also found increasing contributing of NAFLD as cause for chronic liver disease rising from 47% to 76% for the earlier and later series, respectively [31]. These data are consistent with the clinical observation that NAFLD is now the commonest liver disorder seen in liver clinics of Western industrialised countries [4].

2.3. Age and the Prevalence and Severity of NAFLD. The prevalence of NAFLD in the general population increases with age; from 1 to 3% in children [32], 5% in teenagers [32, 33], 18% between 20 and 40 years, 39% in those aged 40 to 50 years, and to over 40% in those greater than 70 years [4, 26, 30, 34, 35]. In general, fatty liver is more prevalent in men than women up to the age of 60 years. Beyond menopause, the prevalence of fatty liver rises sharply in women and exceeds that observed in their male counterparts [5, 13, 36].

There are currently 2 epidemiological studies that have reported on fatty liver in individuals over 70 years. The first recruited 91 inpatients from 3 rehabilitation hospitals in Israel [34], and the second recruited 351 outpatients from a tertiary liver clinic in UK [37]. In the earlier study, Kagansky et al. adopted US and CT scan as diagnostic modality for NAFLD, while Frith et al. used liver biopsies. Both studies found NAFLD prevalence to exceed 40% in individuals over 70 years old. However, Frith et al. found a high prevalence of fibrosis (40%) and cirrhosis (14%) in the liver biopsies of these older individuals, which contradicted findings from Kagansky et al. who found no stigmata of chronic liver disease on clinical examination of their octogenarian

cohort. The weakness of the Kagansky study was reliance on clinical examination for detection of advanced liver disease, which has very low sensitivity especially in the absence of decompensated cirrhosis [38].

2.4. Effects of Overweight, Insulin Resistance, and Impaired Glycemic Control on NAFLD Prevalence. The prevalence and severity of NAFLD is also influenced by presence of metabolic risk factors, such as overweight/obesity and T2D. The prevalence of NAFLD and NASH in T2D are 76% and 22%, respectively [27]. Furthermore, the prevalence of NAFLD correlates with the degree of impaired glucose metabolism, increasing from 27% in subjects with normal fasting glucose (fasting blood glucose, FBG < 6.1 mmol/L), to 43% and 62%, respectively, in those with impaired glucose tolerance (FBG ≥ 6.1 mmol, but < 7 mmol/L) and T2D (FBG > 7.0 mmol/L) [30]. Likewise, the prevalence of NAFLD increases in proportion to body weight category. In the Dionysos study, NAFLD was present in 24.5%, 67%, and 94% of the normal weight, overweight, and obese population, respectively [25, 39]. Pooled analysis of liver biopsy reports in bariatric surgery patients (BMI greater than 40 kg/m^2 or greater than 35 kg/m^2 for those with medical comorbidities) has shown steatosis and NASH prevalence to be 61% and 36%, respectively, whereas fibrosis and cirrhosis are present in 16% and 2%, respectively [4, 25, 26].

However, although NAFLD and NASH are more common in obese patients, it is now recognised that some of these patients do not meet the weight criteria for obesity. Not surprisingly, this is more common among Asian patients (even with ethnic-specific criteria), though it has also been increasingly recognised in Western Countries [4]. In China, for example, 40% of patients with NASH do not meet ethnicity-adjusted BMI for overweight or obesity [40, 41]. However, most patients with NAFLD/NASH whose BMI is within an ethnically adjusted "normal range" can be described as "metabolically obese," where they have increased visceral fat tissue (VAT) and usually have detectable insulin resistance (IR) in spite of normal BMI [4, 36, 42].

3. Ageing Changes in Liver and Other Tissues Relevant to NAFLD/NASH

Age-related cellular and organ system changes are not uniform. In this section, we focus on changes within the liver and the pattern of fat distribution, the latter with respect to metabolic characteristics that are relevant to NAFLD.

3.1. The Ageing Liver. Between the ages of 20 and 70, there is a decline in hepatic blood flow (by 33%), hepatic volume (up to 25%), and liver function [43]. The impact of reduction of liver volume and hepatic blood flow in the elderly is unclear, but it tends to alter the pharmacokinetic profiles of drugs that undergo mandatory hepatic oxidation [44]. The octogenarian liver also has fewer, but larger, hepatocytes, increased polyploidy, and higher binuclear index [44], as well as a reduction in mitochondria numbers. The later may impact on oxidative respiration. Additionally, there is a reduction in bile acid synthesis, with consequent change to bile acid secretion and bile flow. There is an age-related decline in hepatic metabolism of LDL cholesterol, leading to elevated serum cholesterol. The combined effects of changes in bile acid secretion and cholesterol metabolism likely contribute to increased serum cholesterol levels and an increased frequency of gallstones formation.

The ageing liver does appear to be more susceptible to the effects of drugs and other toxins, having diminished regenerative capacity to recover from insults, as evident by an increase in morbidity and mortality in hepatic resections in experimental studies in patients greater than 60 years old [44, 45].

3.2. Age-Related Changes in Body Composition, and Consequences for Metabolic Syndrome. There is an age-linked increase in abdominal adiposity and fat deposition in muscles (skeletal and cardiac), liver, and bone marrow [46]. The loss of lean body (muscle) mass is masked by the increase in total and regional adiposity with ageing. Not surprisingly, anthropometric indices such as body mass index (BMI) and waist circumference do not accurately reflect total and regional adiposity as well as they do in younger patients [46]. Also, the body fat distribution in the elderly is shifted from subcutaneous adipose tissue (SAT) to VAT locations, resulting in deleterious metabolic consequences such as IR [47, 48].

Several studies have now shown increasing prevalence of the metabolic syndrome with advancing age [49, 50]. Utilising data collected from 31,126 adult (>20 years) in NHANES group from 1999 to 2004, Churilla et al. showed an increasing likelihood of developing metabolic syndrome with age. Specifically, the odds ratio of developing metabolic syndrome rises from 1.66 to 5.93 in those aged 30–39 years to 60–69 years, respectively. Beyond this, the odds ratio declined slightly, to 4.39, in those over 80 years old [49].

As discussed before, there is a very strong correlation between metabolic syndrome and the subsequent development of NAFLD conversely, presence of hepatic steatosis is predictive for development of metabolic syndrome [13, 25, 36, 51]. Yamada et al. retrospectively looked at approximately 13,000 individuals undergoing routine health checkup and found that the incidence of T2D was 2.9% in men with fatty liver, compared with 0.6% in men without fatty liver. Similarly, T2D was found in 2.5% of women with fatty liver compared to 0.4% who did not develop fatty liver on ultrasound over the 5-years studied period [42]. In light of these and similar studies, it is not surprising that the age and gender specific prevalence trends for NAFLD mirror those for metabolic syndrome, peaking in middle age, and decreasing in octogenarians [13, 36, 37, 52].

4. Pathogenesis of NAFLD

The pathogenesis of NAFLD and progression to steatohepatitis have not been fully deciphered. In this section, we will briefly discuss what is known so far, with special reference to aspects relevant to interactions with ageing.

An older concept of NASH pathogenesis, the so-called "two-hit" hypothesis of Day and James [58], proposed that

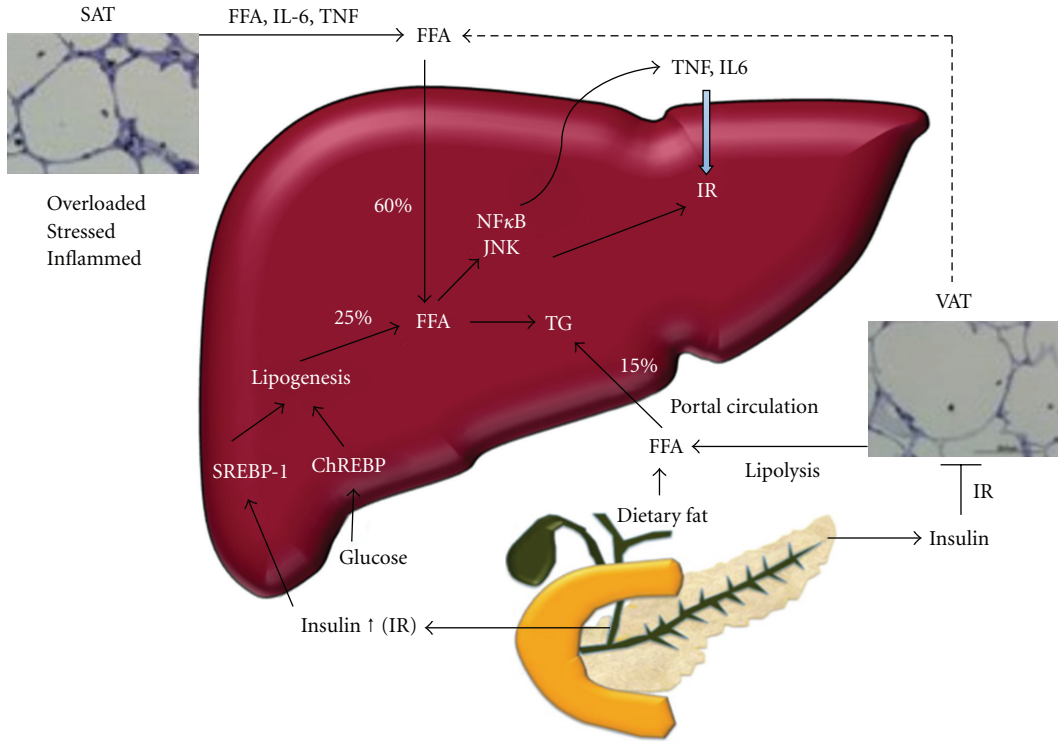

Figure 1: Mechanism of hepatic steatosis, adapted from Larter et al. 2010 [12], showing also interactions between adipose tissue in differing sites with liver in the development of insulin resistance (IR). FFA: free fatty acids; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; TG: triglyceride.

hepatocyte TG accumulation resulting from metabolic imbalance (obesity, IR and diabetes) is what leads to steatosis (the "first hit") and that the lipid-laden liver is then vulnerable to injurious processes ("second hit" insults) such as cytokines and oxidative stress [58, 59]. Damaged and dying hepatocytes and/or recruited and activated inflammatory cells, such as Kupffer cells, generate other signals (cytokines, growth factors, and oxidative stress) which activate scar-forming hepatic stellate cells with resultant development of liver fibrosis and cirrhosis [60, 61]. While this older concept has been useful for focusing attention on proinflammatory and profibrotic mechanisms in fatty liver disease, it does not account for why the majority of cases of simple steatosis do not progress to NASH or cirrhosis and it fails to take into account the cytotoxic and proinflammatory properties of several lipid species. Thus, a more encompassing concept about NASH pathogenesis considers that the profile of lipid molecules in NASH differs from that of simple steatosis, acknowledging that TG is a nontoxic safe storage form of lipid in tissues such as the liver but that other molecules (candidates include free fatty acids (FFA), toxic sphingophospholipids-like ceramide, diacylglycerides (DAG), free cholesterol, and oxysterol metabolites) could mediate tissue injury directly, a process termed "lipotoxicity" [62]. Lipotoxicity is also favoured as the major pathway for pancreatic beta cell injury in T2D [62–66] and as part of the process of atherogenesis and cardiac toxicity with metabolic syndrome [62].

4.1. Mechanism of Steatosis. Accumulation of fat in the liver represents an imbalance in hepatic lipid turnover. The liver plays a pivotal role in lipid metabolism. It takes up circulating free fatty acids and other lipids that arise from intestinal uptake/dietary sources, from lipolysis of peripheral storage sites (adipose tissue) and *de novo* synthesis (lipogenesis). The liver then exports the lipid for storage in adipose stores as triglyceride-rich very low density lipoproteins (VLDL). The mechanisms potentially contributing to hepatic steatosis are summarised in Figure 1.

Steatosis occurs when FFA supply to the liver (from dietary intake, peripheral lipolysis, and *de novo* lipogenesis) exceeds hepatic FFA elimination (via oxidation, re-esterification, and excretion as very low density lipoproteins (VLDL)). These pathways have recently been reviewed and will only be discussed briefly here [11, 12, 26, 67]. Kinetic studies indicate that approximately 75% of hepatic lipids (TAG/TG) in obese patients with NAFLD comes from peripheral sites (60% from nonesterified free fatty acids from lipolysis and 15% from diet), with approximately 25% arising from *de novo* lipogenesis [68]. The latter process is governed by several nuclear transcription factors that are activated by insulin (in the case of sterol regulatory element binding proteins (SREBP)1 and 2) and glucose (in the case of carbohydrate-responsive sterol regulatory element binding protein (ChREBP)1) [11, 12, 69]. Both SREBP1 and ChREBP activate fatty acid synthase (FAS), the rate limiting step in biosynthesis of long chain fatty acids, while SREBP2 regulates

cholesterol biosynthesis. These pathways provide a partial explanation why insulin resistance and premetabolic syndrome (which is hyperinsulinemia and glucose intolerance) are strongly associated with steatosis. A more important contribution may come from dysregulation or "failure" of peripheral adipose tissue storage sites leading to store excess energy as TG, to abnormal lipid partitioning to the liver and other nonphysiological storage sites like muscles [12].

Several studies have clearly indicate that the development of NAFLD and of metabolic syndrome is more closely linked to the pattern of fat distribution than to total body fat. In particular, central (or visceral) adiposity is strongly implicated in the development of both hepatic steatosis and metabolic syndrome [17, 60, 70]. Clinical studies have also highlighted the adverse contribution of visceral adipose tissue (VAT) to the metabolic and liver complications of overweight/obesity [48, 71]. Similarly, in *ob/ob* (leptin deficient) mice, which are hyperphagic, develop insulin resistance and have severe steatosis [48], an adiponectin transgene (which restored normal serum adiponectin levels) expanded subcutaneous adipose and worsened obesity, but improved metabolic indices, such as glycemic control, in association with amelioration of NAFLD [72]. In other murine experiments, using C57B/6 mice, Tran et al. found transplanting subcutaneous adipose tissue (SAT) to a VAT location, or to a lesser extent to another SAT location, improved insulin sensitivity and reduced body weight of recipient animals. This indicates possible intrinsic physiological difference in the adipocytes between the two sites [47].

The deleterious metabolic effect of increased VAT can be partially explained by dysregulation of adipocytokines (TNF-α, leptin, resistin, and most notably adiponectin). This results from increased recruitment of inflammatory cells, particularly macrophages [73] in the setting of stressed and hypertrophic adipocytes caused by overnutrition and obesity [74]. VAT secretes more proinflammatory cytokines (TNF-α, IL-6, and monocyte chemoattractant protein-1 (MCP1)), and this, coupled with direct drainage to the liver via the portal circulation, emphasizes the ability of VAT to directly impair hepatic insulin signaling and promote inflammation. TNF-α can activate both nuclear factor-kappa B (NFκB) and c-jun *N*-terminal kinase (JNK), promoting serine phosphorylation of the insulin receptor substrate which directly impairs insulin signalling. Additionally, MCP-1 can activate inflammatory pathways and promote hepatocyte TG accumulation directly.

Insulin resistance in adipose tissue allows inappropriately sustained lipolysis with release of FFA, which are shunted to the liver at times when the liver is programmed for lipogenesis rather than for fat disposal [66]. Adipose inflammation, coupled with hepatic insulin resistance, is one of many possible connections linking adipocytes and liver in NASH, as addressed next [12].

The ageing process results in increased prevalence of metabolic syndrome and T2D, possibly via preferential fat distribution to VAT sites. These factors all culminate in dysregulated lipid handling by the liver, causing steatosis and partially explaining the resultant progression of NAFLD to NASH.

4.2. NAFLD and Insulin Resistance. Insulin resistance is found in virtually every patient with NASH and in approximately 60% of all patients with NAFLD. The pathogenesis of insulin resistance involves a combination of genetic polymorphisms that influence insulin secretion and many acquired factors, such as sedentary lifestyle, medications, chronic illnesses, ageing, and other environmental factors which promote obesity and immobility [26, 78]. Insulin resistance, whether acquired or genetically determined, raises serum insulin and increases serum free fatty acid (FFA) levels. In the presence of a steatotic liver, the hyperinsulinemic state fails to suppress adipose FFA flux, resulting in these FFA being taken up by the liver, driving TG production and ultimately perpetuating more hepatic steatosis and inflammation when the mechanisms for lipid storage in adipocytes become overwhelmed [10]. As discussed earlier, the accompanying hyperinsulinemia promotes *de novo* hepatic lipogenesis [79], further promoting lipid overload in hepatocytes.

4.3. What Drives Progression to Steatohepatitis (NASH) Once Steatosis Occurs? Significant hepatocyte apoptosis is a feature of NASH and forms the basis of a serum test for caspase3-generated cytokeratin-18 fragments (a biomarker of apoptosis), which is being increasingly used to differentiate between patients with NASH and those with simple steatosis [26, 62, 80]. Hepatocyte apoptosis itself triggers regenerative mechanisms to replace dead hepatocytes. However, aberrant repair in some individuals eventually leads to activation of hepatic stellate cells (HSC) to myofibroblasts and hepatic recruitment of proinflammatory and profibrogenic immune cells to the liver [61].

As liver injury and cell death progresses, fat laden hepatocytes and perisinusoidal fibrosis may impair microvascular hepatic blood flow, causing decreased oxygen and nutrient exchange, and thereby stimulating microvascular inflammatory response and a self-perpetuating cycle of liver damage and vascular insufficiency [26, 81].

These self-perpetuating cycles of inflammation and apoptosis are effected in some ways by adipokines, toxic lipid species, mitochondrial dysfunction, vascular disturbance, and possibly gut bacterial endotoxins [26]. Ageing can alter some of these modulators, such as by changes in SAT/VAT distribution with its effect on adiponectin levels [82], reduced liver blood flow, and reduced ability of ageing liver to adapt to injury. Such changes could contribute to worsened liver histology of NAFLD in older people.

5. Progression of NAFLD: Lessons from Natural History Studies

Although simple steatosis is generally nonprogressive [83], the initial assessment of SS as always being benign is not fully supported by current evidence [53–56]. Documentation of progressive fibrosis is problematic because serial liver biopsies are necessary and there remains the possibility of sampling error. Table 1 summarizes 4 early studies using serial liver biopsies to assess disease activity and progression in NAFLD/NASH. In essence, the studies have shown progression in disease activity (SS to NASH, NASH to fibrosis,

Table 1: Disease progression in NAFLD/NASH and risk factors predicting disease progression [53–56].

	Powell et al. 1990 [53]	Harrison et al. 2003 [54]	Fassio et al. 2004 [55]	Adams et al. 2005 [56]
Number of patients with serial liver biopsy	13	22	22	103
Age (years)	49 (16–70)	50.6 (33–64)	45 (20–69)	45 (19–65)
Biopsy interval (years)	1–9	N/A	1–3	0.7–21
Follow-up period (years)	1.5–21.5	1.4–15.7	3–14.3	N/A
Disease activity (%)				
Unchanged	46%	50%	68%	34%
Progress	30%	32%	31.8%	37%
Regress	23%	18%	0%	29%
Risk factors associated with NASH progression	None identified	AST	Obesity BMI	Obesity BMI Low initial fibrosis score

N/A: not available; AST: aspartate transaminase.

and fibrosis to cirrhosis) in up to a third (30–37%) of patients, while a quarter (23–29%) shows the reverse, that is, histological improvement. Both obesity and BMI were predictive of disease progression [55, 56]. Additionally, more severe grades of baseline steatosis, elevated serum alanine aminotransferase (ALT), platelet count, and weight gain greater than 5 kg were other predictors of disease progression [53, 54]. However, none of these studies looked at patients greater than 70 years old.

What is clear in NAFLD is that once advanced fibrosis has developed, the risk for hepatocellular carcinoma (HCC) is about 5–7%. If the person is cirrhotic at time of diagnosis, the risk of developing portal hypertension as a major complication is also high; 17%, 23%, and 52% at 1, 3, and 10 years, respectively [84].

Many cross-sectional studies have sought predictors of hepatic fibrosis in NAFLD. Relevant factors include age (especially over age 50 years), BMI > 28–32 kg/m^2, insulin resistance or T2D, and raised serum ALT [13, 14, 27, 57, 85–91]. In multivariate analysis, age, BMI, arterial hypertension, ALT, insulin resistance, and hepatic necroinflammatory grade were shown to independently predict presence of fibrosis [27, 36, 57, 86, 87, 89, 91].

The other key aspect of clinical outcome studies in NAFLD is the increased likelihood of death from cardiovascular diseases (coronary heart disease, stroke) and nonhepatic malignancy. However, liver-related mortality ranks third in the causes of death. The risk of liver-related death is even higher in the subgroup of patients with NASH compared to those with SS (2–10% for NASH versus 0–2% for SS) (Table 2) [57, 75–77].

6. Management of NAFLD in the Elderly

The cornerstone of the management of NAFLD is to correct the disturbed metabolic milieu by encouraging an active lifestyle so as to counteract increases in body weight and improve insulin sensitivity. Further, treatment of coexisting metabolic disorders like hypertension, dyslipidaemia, and glucose intolerance/diabetes is important in the overall management plan.

6.1. Lifestyle Changes. While lifestyle changes are widely promoted, adherence remains a major issue. A program of cognitive behavioural therapy remains the most effective tool to obtain long-term adherence. In these programs, individuals are educated to self-manage their diet and to undertake moderate daily physical activity [92, 93]. Compliance to modest caloric restriction and increased physical activity result in both sustained weight loss and improved cardiorespiratory fitness, the latter assessed by the peak oxygen consumption (VO_2 max). In one study, a 21% improvement in physical performance measures was observed in the diet-exercise group as compared to 12% and 15% in the diet alone and exercise alone groups, respectively [94].

Weight loss of only 5–10% (e.g., 3.7 kg in a 75 kg man) decreases liver fat by 40% in both nondiabetic and diabetic subjects [60, 95]. Moreover, aerobic exercise reduces hepatic fat content independent of weight loss [96]. The beneficial effects on hepatic steatosis are likely a consequence of increased insulin sensitivity through reduced peripheral lipolysis, inhibition of lipid synthesis, and stimulation of FA oxidation [97]. On the other hand, any effects on hepatic inflammation and fibrosis have been inconclusive [98]. In individuals aged over 65 years, the beneficial effect of diet and exercise on physical fitness, muscle strength [99], and *metabolic* fitness as shown by reduction in hepatic steatosis, serum cholesterol, high blood pressure, and improved insulin sensitivity Shah et al. [99] have all been confirmed. Further studies on the efficacy of such interventions in clinical and histological outcomes in NAFLD/NASH will be of interest.

6.2. Pharmacotherapy—The Insulin Sensitisers. Currently, there are no approved drugs for use in the treatment of fatty liver or NASH. As insulin resistance is central to NAFLD, agents that improve insulin sensitivity appear promising. To date, two classes of insulin sensitising agents, metformin, and thiazolidinediones (TZDs) have been evaluated.

Table 2: Natural history data on NAFLD.

	Adams et al. 2005 [57]	Ekstedt et al. 2006 [75]		Ong et al. 2008 [76]	Rafiq et al. 2009 [77]	
	NAFLD	NASH	NNFL	NAFLD	NASH	NNFL
N	435	71	58	817	72	101
Age at diagnosis (in years)	49 ± 15	55 ± 12	47 ± 12	17+	51 ± 13	49 ± 15
Males/females	213/222				30/70	47/53
Study period	1980–2000	1988–1993		1988–1994	1979–1987	
Followup (in years)	7.6 ± 4	13.7 ± 1.3		8.4 (median)	10.5 (median)	13.0 (median)
Advanced cirrhosis	13 (3.1%)	7 (9.8%)	0			
HCC	2 (0.5%)	2 (2.8%)	0			
Deaths (total)	53 (12.1%)	26 (20.3%)		80 (9.7%)		
IHD	13 (2.9%)	11 (15.5%)	5 (8.6%)	20 (2.4%)	7 (12.3%)	15 (20.3%)
Non-HCC cancer	15 (3.4%)	4 (5.6%)	1 (1.7%)	19 (2.3%)	5 (8.8%)	9 (12.2%)
Liver*	7 (1.6%)	2 (2.8%)	0	5 (0.6%)	10 (17.5%)	2 (2.7%)

NNLF: non-NASH fatty liver (which equates with simple steatosis referred to in the text); *liver related mortality.

Metformin causes weight reduction and improves insulin sensitivity by decreasing hepatic glucose output and increasing peripheral glucose uptake, reducing hepatic lipogenesis and increasing hepatic fatty acid β-oxidation (by activation of AMP-activated protein kinase), and suppression of lipogenic transcription factor, SREBP-1 [100]. Early studies of metformin in NASH showed significant reduction in hepatic steatosis and ALT, but histologic follow-up data are scarce and have not shown improvement in hepatic necroinflammatory grades [101–103], except in one study where it correlated with the degree of weight loss [101]. The use of metformin in the elderly could be also problematic because of the additional risk of severe life-threatening lactic acidosis (5 per 100,000 prescriptions) [104]. Currently, metformin use for NASH is not recommended [100, 105].

Thiazolidinediones are PPAR-γ agonists that exert insulin-sensitizing actions on adipocytes and in the liver. PPAR-γ agonists increase adipocyte numbers, promote their differentiation, and facilitate uptake and storage of FFA, thereby reducing ectopic fat deposition in liver and muscles and restoring insulin sensitivity. TZDs also increase serum adiponectin levels, with improved insulin sensitivity. In NAFLD, the TZDs have been shown to reduce serum ALT levels and hepatic steatosis, with some effects on necroinflammation activity, but effects on hepatic fibrosis have not usually been observed; this could relate to short duration of use, typically 6–12 months [106–109]. More recently, Ratziu et al. extended duration of rosiglitazone for an additional 2 years in 53 patients with liver biopsy-proven NASH. Disappointingly, even though rosiglitazone substantially improved steatosis in the first year, longer treatment did not improve NASH histology despite maintained effects on insulin sensitivity and ALT levels [110]. In general, TZDs used to treat NASH have been well tolerated, but significant weight gain (2–6 kg) predominantly in the SAT area occurs in up to 72% of patients and this is a concern. Also, with regards to the older patient with NAFLD, TZDs can precipitate heart failure and their use is not recommended for patients with New York Heart Association Class III and IV heart failure [67, 111]. Rosiglitazone has been withdrawn from the European market due to increased rates of myocardial infarction, but not overall cardiovascular mortality in a meta-analysis of approximately 35,000 patients from 56 randomised controlled trials [112]. Its access is restricted in Australia and America [113]. Pioglitazone appears to be the safer alternative. Troglitazone has been withdrawn worldwide due to significant fatal hepatotoxicity.

6.3. Bariatric Surgery. Bariatric surgery is currently recommended for morbidly obese patients (BMI > 40 kg/m^2) and for metabolic syndrome or T2D when BMI exceeds 35 kg/m^2 [114]. Earlier approaches to obesity included jejunoileal bypass surgery, which was abandoned owing to unacceptable risks of liver failure. All other types of bariatric surgical techniques can decrease excess body weight by up to 50%, with generally less weight loss in laparoscopic procedures versus more invasive approaches [115–118]. This weight loss improves insulin sensitivity and reduces the frequency and severity of metabolic syndrome, diabetes and its complications [97]. With respect to NAFLD/NASH, follow-up liver biopsies have shown a reduction in hepatic necroinflammatory activity including resolution of NASH in the majority of cases and reduced hepatic fibrosis in some [119, 120]. Bariatric surgery in patients aged greater than 60 years old is associated with significantly increased overall morbidity (19%) compared with those less than 60 years (11%). However, with the exception of a small subset of patients with significant heart disease, there is no significant difference in the observed-to-expected mortality ratio [121]. Therefore, bariatric surgery can still be considered reasonable in carefully selected older patients, given its significant metabolic benefits.

6.4. Is There a Role for Liver Transplantation for NASH Cirrhosis in the Elderly? Currently, 5–10% of liver transplants in the USA are for patients with NASH-related cirrhosis, while NASH is becoming an important predisposing factor for HCC. Because liver transplantation for hepatitis C peaked at 28% in 2002 and has remained stable since [31], it has been projected that NAFLD will become the most common

indication for liver transplantation in the next 20–30 years [122]. The obesity epidemic affects not only recipients, but also potential organ donors, up to a quarter of whom have hepatic steatosis. The prevalence of steatosis in donor livers increases with BMI and age. Fatty change in the donor liver is associated with higher rates of primary graft nonfunction, likely due to the increased severity of hepatic ischemia-reperfusion injury [81].

Optimizing body weight before transplantation is rarely achieved and may be hazardous in patients with decompensated cirrhosis. Additionally, morbidly obese patients carry a high perioperative risk and are at increased risk for recurrence of progressive fatty liver disease after transplantation, especially in the setting of immunosuppressive therapy; hepatic steatosis occurs in up to 60% of transplant recipients, with 5–10% progressing to cirrhosis and graft loss [122]. Finally, metabolic syndrome is among the most common causes of death after all liver transplants and is likely to be accelerated among patients with NASH as the original cause of endstage liver disease.

The mean age for liver transplantation increased from 29 in 1985, to 41 in 1995. By 1999, 21% of liver transplant recipients were in people aged 60 years or older [123, 124]. Studies have also shown that, with careful selection, there are no differences in survival or length of hospital stay for individuals over 60 years old compared to their younger counterparts [124]. Therefore, liver transplantation may be a possible option in carefully selected patients with NASH cirrhosis.

7. Conclusions

Disease incidence, severity, and progression in NAFLD/NASH are strongly associated with presence of components of metabolic syndrome, in particular diabetes and obesity, and are also governed by the interactive effects of both genetic background, sex, age, and environmental factors (food intake, level of physical activity). Advanced age is associated with disease severity and fibrosis progression; a relatively high proportion of individuals with progressive forms of NAFLD develop cirrhosis by the time there are in their 70s or beyond, although more data are required on the exact risks. Advanced forms of NAFLD (NASH and cirrhosis) are associated with increased standardised mortality and a relatively high risk of liver-related deaths.

NASH is associated with increased risk of death from cardiovascular disease and nonliver malignancies, as well as from liver complications. The practical implication is that clinicians need to consider early interventions to optimise the management of modifiable metabolic risk factors, like glycemic control in T2D, hypertension, and dyslipidemia, each of which could also contribute to disease progression in NAFLD. For all patients with NAFLD, the cornerstone to management remains correction of modifiable risk factors. Exercise and dietary restriction can be very effective in carefully selected patients and should be used in a multidisciplinary approach, involving physiotherapists, dieticians, and occupational therapists to overcome potential physical limitations in older patients, such as osteoarthritis or decreasing mobility from other causes. The insulin sensitisers are not approved for use in this country for treatment of NASH, and they should be used with caution in elderly people given the increased likelihood of coexisting medical conditions like congestive heart failure.

Decompensated NASH cirrhosis may become the number one indication for liver transplantation in the next two decades. This means that patients being considered for transplantation are likely to be much older and have longstanding medical comorbidities related to metabolic syndrome. These need to be assessed and managed prior to acceptance onto a transplantation list. Transplant physicians may need to manage weight loss prior to transplantation of the obese and morbidly obese, but also will be required to manage diabetes, osteoporosis, hypertension, dyslipidemia, immunosuppression, and the challenge of polypharmacy from prescriptions these elderly population will inevitably require.

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Review Article

Liver Regeneration and Aging: A Current Perspective

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Many organ systems exhibit significant age-related deficits, but, based on studies in old rodents and elderly humans, the liver appears to be relatively protected from such changes. A remarkable feature of the liver is its capacity to regenerate its mass following partial hepatectomy. Reports suggests that aging compromises the liver's regenerative capacity, both in the rate and to the extent the organ's original volume is restored. There has been modest definitive information as to which cellular and molecular mechanisms regulating hepatic regeneration are affected by aging. Changes in hepatic sensitivity to growth factors, for example, epidermal growth factor (EGF), appear to influence regeneration in old animals. Studies have demonstrated (a) a 60% decline in EGF binding to hepatocyte plasma membranes, (b) reduced expression of the hepatic high affinity EGF receptor and (c) a block between G1 and S-phases of the cell cycle in old rats following EGF stimulation. Recent studies suggest that reduced phosphorylation and dimerization of the EGF receptor, critical steps in the activation of the extracellular signal-regulated kinase pathway and subsequent cell proliferation are responsible. Other studies have demonstrated that aging affects the upregulation of a Forkhead Box transcription factor, FoxM1B, which is essential for growth hormone-stimulated liver regeneration in hepatectomized mice. Aging appears to compromise liver regeneration by influencing several pathways, the result of which is a reduction in the rate of regeneration, but not in the capacity to restore the organ to its original volume.

1. Introduction

On the one hand, the liver, unlike most other organs, does not exhibit well-documented or marked changes in either structure or function during the aging process (see [1–4] for reviews). There have been few comprehensive studies on liver morphology during aging; most have been performed using rodent models and have been qualitative in nature. Studies using human liver tissue have suffered from dependence on postmortem samples or on samples from subjects diagnosed with liver disease. There is quantitative evidence that hepatocytes in males of one inbred rat strain (Fischer 344) increase in volume through maturity and, subsequently, become smaller such that the size of cells in immature and senescent animals is equivalent [5]. Other changes in hepatocellular structure include (a) a loss of smooth surfaced endoplasmic reticulum, (b) an increase in the volume of the dense body compartment, for example, secondary lysosomes, residual bodies, or lipofuscin, and (c) an increase in hepatocyte polyploidy (see [6] for a review). None of these age-related changes is manifested in significant declines in hepatic function(s).

On the other hand, there are data that demonstrate specific age-related changes, including a loss of hepatic volume and a decline in hepatic perfusion; both of which may affect certain liver functions, such as first pass pharmacokinetics [7, 8]. However, data from clinical liver function tests are inconclusive and fail to identify significant age-associated deficits in hepatic functions ([9, 10], see [2] for a review). Several studies, including our own, have demonstrated moderate age-related changes in biliary function, including decreased bile flow and bile acid secretion ([11], see [3] for a review).

The clearance of drugs that undergo mandatory Phase I hepatic metabolism may be compromised in the elderly (see [12] for a review). However, there is little evidence to support the hypothesis that reduced hepatic drug clearance reflects concomitant declines in the amounts or efficacies of human liver cytochrome P-450 isoforms [13]. A recent review argues

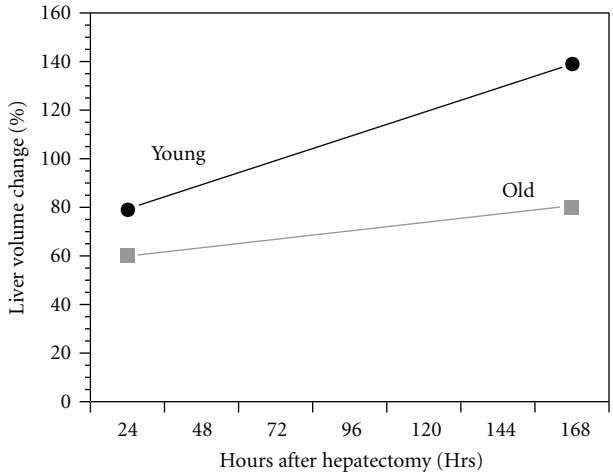

Figure 1: Effect of animal age on liver volume at two intervals following partial hepatectomy in young and old rats. Data derived from [15].

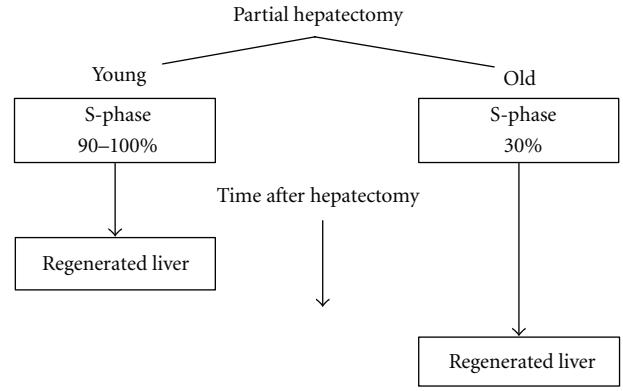

Figure 2: Effect of age on the number of hepatocytes entering S-phase and the rate of liver regeneration following partial hepatectomy.

that substantial age-related declines occur in hepatic Phase I drug metabolism as evidenced by reduced clearance of high-clearance drugs and that other data are misleading since most studies have assessed protein-bound plus free-drug concentrations (total clearance) rather than separating these two components [14]. It should be noted that drug clearance reflects several variables, including volume of distribution, protein binding, intrinsic hepatic metabolism, and renal clearance. While there are data suggesting that certain low-protein binding drugs exhibit reduced clearance in the elderly; the evidence for a similar decline in free-drug clearance is less viable. Studies by us and by others assessing the efficacy of specific P-450 isoforms across a broad age spectrum in humans suggest that intrinsic cytochrome P450-mediated hepatic drug metabolism, not total drug clearance, remains unchanged in humans as a function of age [12, 13]. The question of whether or not liver functions are compromised in senescent animals or elderly humans remains unclear. The late hepatologist, Hans Popper, stated that "aging exerts a limited effect on the constitutive functions of the liver and more on its response to extrahepatic factors…" [14].

Perhaps a more clinically significant age-related change is a marked decline in the rate of hepatic regeneration following partial resection (hepatectomy) or chemically induced injury. This is manifested as a delay in hepatocyte proliferation following hepatectomy and is documented by a number of studies, including that of Popper [16] (Figure 1). The purpose of this brief review is to present a perspective of the current understanding of the cellular and molecular factors and mechanisms that contribute to the diminished hepatic regeneration rate in old-animal models and in elderly humans.

2. Basics of Liver Regeneration

First, hepatocytes constitute a population of highly differentiated, quiescent, yet intermitotic cells with few cells undergoing division at any one time, for example, approximately 1 mitotic figure per 20,000 cells in the resting liver. Second, the normal regenerative process reflects a global hyperplasia, that is, compensatory growth and division of existing hepatocytes, rather than a cellular hypertrophy or a primary stem cell response. Third, the regenerative process is highly regulated by signal transduction pathways (see [17] for a review). Fourth, the initiation of hepatocyte proliferation or liver regeneration requires the activation of specific cell cycle and mitogenic genes, as well as the repression of those genes responsible for inhibiting hepatocyte proliferation in the resting organ. The consensus is that fewer hepatocytes in senescent animals and elderly humans enter S-phase after partial hepatectomy in comparison to younger subjects, and those that do so less rapidly and that this age-related delay compromises the rate of liver regeneration (Figure 2).

3. Why the Concern about Compromised Liver Regeneration in the Elderly?

One reason is the marked increase in mortality due to liver disease in elderly subjects in comparison to younger populations. Regev and Schiff reported 3–5-fold increases in deaths due to liver diseases in the over 65 population versus those under 45 years of age [18]. Another basis for concern is the increased demand for donor livers for transplantation. This issue is complex since there is an effort to increase the age limits for liver donors and, to some extent, of liver recipients. On the one hand, there is evidence that livers from older donors may be less viable than those from young donors [19, 20]. Recipient age should also be a consideration since Fortner and Lincer reported that post-transplant mortality increases by 15% between 55 and 75 years of age [21]. On the other hand, there are data that suggest that the impact of age is modest with respect to recipient and graft survival, at least during the first few post-transplantation years. For example, both recipient and graft survival rates decline in elderly patients receiving livers from old donors by only 10–15% over the first three post-transplant years [22–24].

In summary, aging does impair liver regeneration with respect to the rate of hepatocyte proliferation following

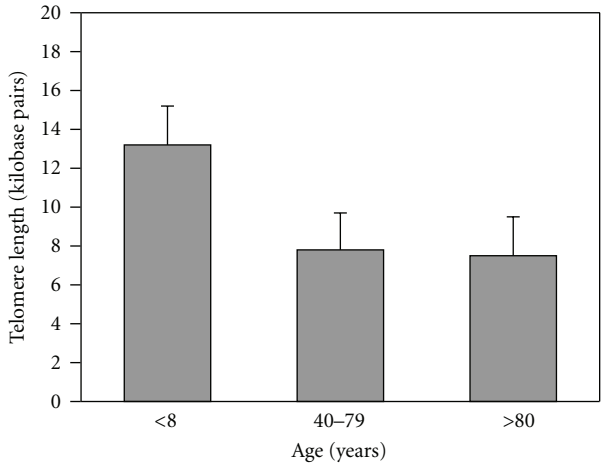

Figure 3: Effect of aging on telomere length in human hepatocytes. Both Takubo et al. and Aikata et al. (not shown) determined hepatocyte telomere length to be between 5–10 kbp in humans 80 years of age. Data derived from [26, 27].

resection. The magnitude of this impairment does not seem to be excessive and, furthermore, may not be an impediment to the use of livers from elderly donors. However, before we can assess this option definitively, we need to clearly understand the cellular and molecular mechanisms that compromise liver regeneration in the elderly. There have been a number of hypotheses, some of which are discussed below. Perhaps the most comprehensive studies on this subject have been performed by Timchenko's group (see [17] for a review).

4. Age-Related Increases in Reactive Oxygen Species, Hepatocellular Residual Bodies, and Lipofuscin

One suggestion is that the documented age-related increase in residual bodies or lipofuscin in hepatocytes reflects an inability to eliminate cellular waste products and that this accumulation compromises normal cell activities (see [25] for a review). Our quantitative electron microscopic analysis demonstrated a 3-4-fold increase in the volume of this intracellular compartment during aging in rats [5]. However, since this compartment accounts for only about 1% of the total intracellular volume of hepatocytes, it seems inappropriate to assign this particular age-related shift a significant role in impeding hepatocyte proliferation and liver regeneration.

Reactive oxygen species (ROS) have been considered a causative factor responsible for a number of pathophysiological changes during aging. A recent study by Haga et al. implicates increased expression/phosphorylation of the adapter protein $p66^{Shc}$ in the enhanced generation of ROS and in initiating apoptosis in hepatocytes after partial hepatectomy in aged mice, but not in the livers of young animals [29]. In this study, hepatocyte proliferation in both young and old cohorts was similar, but cell growth was impaired only in the old mice. Furthermore, ablation of $p66^{Shc}$ diminished posthepatectomy oxidative stress and apoptosis in aged mice, suggesting that this age-associated protein may play a critical role in inhibiting the hepatic regenerative capacity in old animals.

Gielchinsky et al. recently reported an interesting observation that may have clinical ramifications for enhancing hepatic regeneration in the elderly, at least in elderly women [32]. These investigators reported that (a) the post-hepatectomy regenerative rate was restored in aged pregnant mice in comparison to their age-matched, nonpregnant cohorts and (b) this regeneration was achieved primarily by hepatocyte hypertrophy rather than by cell proliferation, the process responsible for normal liver regeneration. The clinical potential resides in the possible pharmacological activation of an important mediator of hepatocyte growth, the Akt/mTPRC1 pathway, and the subsequent switch from a cell proliferative to a cell growth response.

5. Age-Related Loss of Telomere Length

Another hypothesis suggests that an age-related reduction in hepatocyte telomere length results in diminished cell mitosis and apoptosis and, thus, a decline in cell proliferation. For example, Takubo et al. demonstrated a marked age-associated loss in hepatocyte telomere length in humans, and these data were confirmed independently by Aikata et al. [26, 33] (Figure 3). Takubo et al. also reported that the rate at which telomere shortening occurred was markedly higher in hepatocytes in comparison to most other epithelial cell types with high turnover rates, for example, enterocytes and esophageal epithelium [26]. A recent review suggests that the yearly reduction rate in human hepatocyte's telomere length ranges between 55 and 120 base pairs [27].

Obvious changes in cell structure are not always reflected in concomitant functional alterations. Using a telomere restriction fragment deficient mouse model, Denchi et al. demonstrated that the loss of telomere integrity did not compromise liver regeneration following partial hepatectomy [35]. Although the hepatocytes enter S-phase, subsequent mitosis, anaphase, and telophase did not occur. This paper is of particular interest since it demonstrates that mouse hepatocytes subjected to the deletion of a telomere protection protein, TRF2, exhibit frequent telomere fusions, but no evidence of apoptosis or loss of hepatic function(s). Furthermore, post-hepatectomy regeneration was not compromised, but was accomplished via increased cell growth yielding polyploid cells, perhaps indicative of a switch from a proliferative to a cell growth pathway [32].

Interestingly, our stereological analyses showed that mean hepatocyte volume in male F344 rats decreases between 20 and 30 months of age such that cells in the livers of the oldest animals were similar in volume to those in very young animals [5]. Our data also demonstrated that the relative number of binucleate hepatocytes, the nuclear numerical density, and the nucleocytoplasmic volume ratio were similar in the youngest and oldest rats. However, these studies were performed on resting hepatocytes, and the data do not preclude the possibility that small hepatocytes in

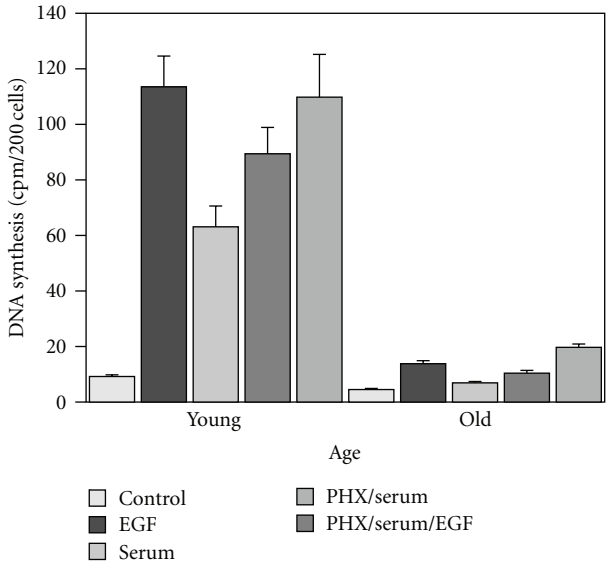

Figure 4: Effect of age on hepatocyte response to EGF and serum growth factors in young and old resting and posthepatectomy rats. Note that the posthepatectomy responses to both EGF (PHX) and EGF/serum were significantly greater in the young rats in comparison to their older cohorts. Data derived from [28].

senescent rats lack the capacity to undergo hypertrophy in response to mitogenic factors.

The recent observation that rejuvenating telomerase activity in a telomerase-deficient mouse model reversed certain well-documented age-related deficits may lend credence to further studies on liver regeneration in this model [36]. The caveat, however, is that aging in this particular telomerase-deficient mouse model may not correctly reflect normal human aging. A recent review by Hoare et al. provides a comprehensive discussion of the relationships between aging, hepatocyte telomere shortening, and hepatic injury or disease [37].

6. Effect of Aging on the Hepatocellular Response to Growth Factors

Twenty years ago, Sawada demonstrated that the hepatocyte proliferative response to EGF was markedly greater in young rats in comparison to old animals and suggested that aging impaired the responsiveness of the cells in old rats to growth factors [28] (Figure 4). These studies provided impetus to the controversy concerning the impact of aging on the hepatocyte proliferative response to growth factors. For some years, researchers have suspected that aging impairs specific growth-regulating molecules and/or their receptors, which, in turn, compromises the regenerative response. Despite Sawada's observation that old hepatocytes did not respond to EGF stimulation as well as did liver cells from young animals, they also reported that there were no age-related losses in either the number of hepatocellular EGF receptors or in their binding affinity. However, Marti, in our laboratory, demonstrated a 60% age-related decline in EGF binding to hepatocyte plasma membranes in rats [38]. Interestingly, Ishigami et al. almost simultaneously reported the absence of any age-related change in hepatocyte EGF binding capacity, but did report a marked decline in EGF-induced DNA synthesis [39]. It should be noted that Ishigami et al. used primary hepatocyte cultures, whereas Marti et al. used hepatocyte plasma membranes isolated from intact livers. The preparation of primary hepatocyte cultures involves the use of collagenase and other enzymes that cleave hepatocyte surface proteins nonspecifically, for example, EGF receptors, resulting in cells from both young and old donors expressing equivalently diminished numbers of receptors. The isolation of hepatocellular plasma membranes does not employ enzymes, and the inherent number of receptors and, assumedly, their affinity for their ligand(s) remain intact during this procedure. Interestingly, we observed an 80% age-related decline in the amount of radiolabeled EGF associated with rat hepatocyte nuclei [40]. Furthermore, Ohtake et al. reported age-related losses of the hepatocyte high-affinity EGF receptor as well as in the level of receptor phosphorylation, a critical step in EGF activation [41].

Several studies have reported diminished activation of a hepatocyte extracellular receptor kinase (ERK) in old rodents in comparison to young animals following partial hepatectomy [30, 31]. This decline leads to reduced EGF receptor phosphorylation and, subsequently, to decreased binding of the adapter protein, Shc, to the receptor, a critical event in the EGF-induced hepatocyte proliferation pathway (Figure 5). Subsequent studies by Kamat and others focused on the molecular pathways that regulate hepatocyte proliferation [34]. These investigators reported significant age-related declines in the expression of hepatocyte EGF receptor mRNA and protein, as well as in EGF receptor phosphorylation and the subsequent activation of ERK (Figures 6 and 7).

Growth hormone (GH) is another mitogenic factor that has been implicated in hepatic regeneration. Krupczak-Hollis et al. reported that GH treatment of old, partially hepatectomized rats enhances hepatocyte proliferation in comparison to similarly aged, nontreated cohorts [42]. Furthermore, the endogenous hepatocellular levels of GH and its receptor decline with age, whereas the level of cyclin D_3, which activates C/EBPα (CCAAT/enhancer-binding proteins) phosphorylation, increases. This phosphorylation enhances C/EBPα complexing with (a) a retinoblastoma gene product, (b) a chromosomal remodeling protein (Brm), and (c) a histone deacetylase to yield an inhibitor of a transcription factor required for hepatocyte proliferation, the Forkhead Box gene, FOXM1B.

The importance of transcription factors in the liver regeneration process has been illustrated in a series of studies by Wang and associates delineating the critical role played by the FOXM1B gene in hepatocyte proliferation [43, 44]. Using a mouse model deficient in FOXM1B, these investigators showed that adenovirus transfection with FOXM1B restored the liver regenerative capacity in mature animals to a level that exceeded that measured in young adult mice. Nontransfected FOXM1B-deficient mice did not exhibit enhanced hepatocyte proliferation.

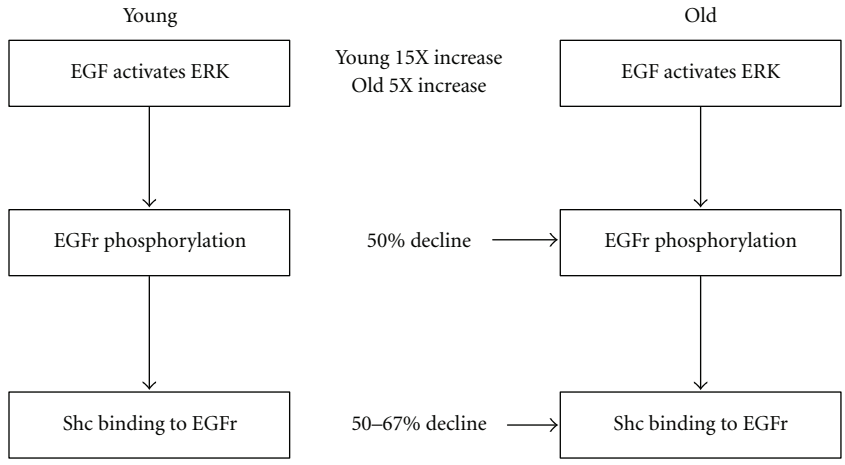

Figure 5: Effect of age on hepatic EGF receptor activation. Data derived from [30, 31].

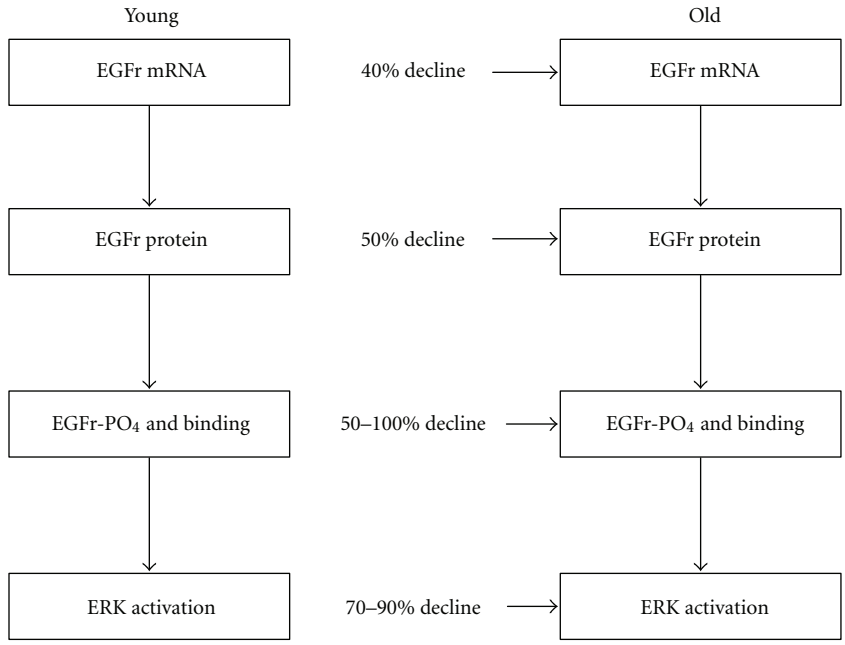

Figure 6: Effect of age on liver EGF activation, phosphorylation, and subsequent activation of the extracellular receptor kinase (ERK). Data derived from [34].

In the resting liver, the hepatocellular levels of cyclin D_3 and C/EBPα are high, thus inhibiting hepatocyte proliferation. In senescent animals, the cyclin D_3 level remains high, activating the phosphorylation of C/EBPα and enhancing the formation of the larger proliferation inhibitory complex. However, following partial hepatectomy in young adult animals, the cyclin D_3 level drops, as does the level of the inhibitor, C/EBPα, permitting the expression of essential transcription factors, for example, FOXM1B and cell cycle genes, and, ultimately, rapid hepatocyte proliferation (Figure 8).

Recently, Chen and colleagues identified a mechanism that regulates FOXM1B transcriptional activation and the liver regeneration process [45]. These researchers showed that the farnesoid X receptor (FXR), a transcription factor that regulates a variety of metabolic pathways, is critical for liver regeneration since FXR-deficient mice exhibit a diminished regenerative capacity. In addition, FOXM1B was identified as a direct FXR target gene, and diminished FXR binding to FOXM1B may contribute to decreased hepatocyte regeneration in the elderly.

7. Other Possible Causes of Diminished Regeneration

A series of studies by Le Couteur et al. reported marked age-related changes in the structure of the hepatic sinusoidal endothelium, including a loss of fenestrae and a thickening

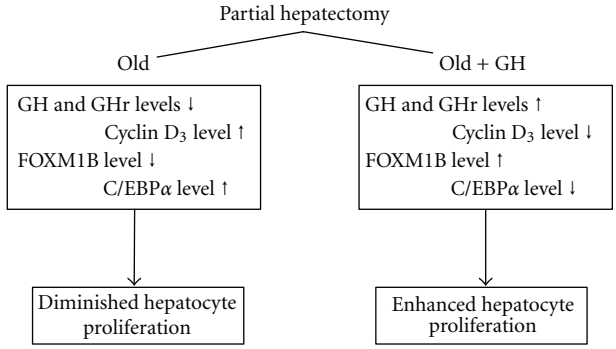

Figure 7: Effect of age on the efficacy of growth hormone induced activation of the pathway resulting in hepatocyte proliferation. Data derived from [34].

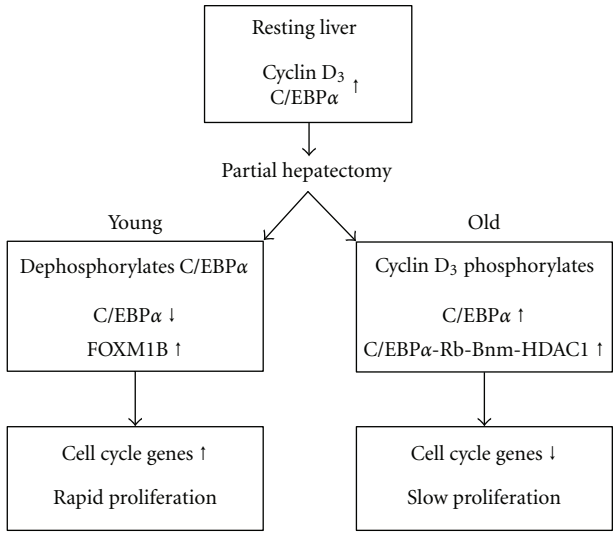

Figure 8: Proposed effect of aging on the molecular factors that regulates hepatocyte proliferation following partial hepatectomy.

of the endothelial cells, a process referred to as pseudocapillarization [46–48]. Interestingly, a very recent paper by Furrer et al. has suggested that pseudocapillarization contributes to the age-related decline in the regenerative response in a posthepatectomized murine model [49]. These investigators demonstrated enhanced liver regeneration in old mice following treatment with a serotonin receptor agonist, and this response correlated with an increase in the number of endothelial cell fenestrae. Their data suggest that the serotonin receptor agonist enhances systemic vascular endothelial growth factor (VEGF) availability, which, in turn, regulates endothelial cell fenestrae diameters, thus improving hepatic perfusion and restoring the hepatic regenerative capacity. However, evidence for or against age-related declines in serotonin and/or VEGF receptors will require definitive ligand-binding studies.

In summary, there are several conclusive statements and a few evidence-based speculations concerning the effect of age on the process of liver regeneration that warrant consideration, including the following.

(i) Liver regeneration is compromised in old animals and in elderly humans.

(ii) The rate of liver regeneration, rather than the regenerative capacity, is diminished in the elderly.

(iii) The induction of hepatocyte proliferation factors and the expression of cell cycle genes is inhibited in the elderly.

(iv) The repression of cell proliferation and cell cycle gene inhibitors is compromised in the elderly

(v) The relative efficacies of normal, cell-cycle-induced hepatocyte proliferation versus an independent pathway involving hepatocyte hypertrophy in maintaining liver functions requires additional study.

(vi) The roles of VEGF, serotonin, and liver sinusoidal pseudocapillarization require further investigation.

Most evidence supports the concept that the age of the liver donor or recipient exerts only a modest impact on post-transplantation patient's survival. These studies suggest that a pretransplantation regimen of growth factors in potential elderly liver recipients merits further consideration.

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Review Article

Clinical Pharmacology of Chemotherapy Agents in Older People with Cancer

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Populations around the world are aging, and the associated increase in cancer incidence has led to the recognition of the importance of geriatric oncology. Chronological age is a poor determinant of pharmacological response to cancer chemotherapy agents. Age-associated changes in physiology and organ function have a significant impact on the clinical pharmacology of cancer chemotherapy agents used in cancer treatment. Altered response to medicines in older people is a consequence of changes in body composition, organ function, concomitant pathophysiology, multiple medications, genetic determinants of drug response, and patient's clinical status. These issues highlight the need to individualize the management of cancer in the older people with consideration of age-related changes in the clinical pharmacology of cancer drugs, analgesics, and adjunctive therapies.

1. Introduction

The continued growth in the proportion of older people in many developed countries has resulted in a large number of older adults with cancer. Cancer is now the second most common cause of death, and approximately half of all cancers occur in the population aged 65 years and older [1]. There is an increasing number of older patients who require the management of cancer. Understanding and defining the optimum use of cancer chemotherapy agents in older cancer patients in the adjuvant, curative, and palliative settings is a very pressing and timely issue in oncology. However, until recently, the area of geriatric oncology has been relatively ignored and there has been less research undertaken than in other areas of oncology which has resulted in a lack of evidence-based guidelines for the treatment of older cancer patients. A significant challenge in providing cancer care to older people is that aging is an individualized and heterogeneous process with variable effects on the clinical pharmacology of drugs used in the management of cancer. This has important implications for choosing the optimal drug regimen, selecting a safe and effective initial, dose and undertaking appropriate monitoring strategies in managing older people with cancer.

The aim of this paper is to identify and discuss the factors which affect the clinical pharmacology of chemotherapy agents in older people with cancer and the medical management of these patients.

2. Factors Affected the Clinical Pharmacology of Chemotherapy in Older Cancer Patients

2.1. Age-Related Changes in Clinical Pharmacology

2.1.1. Pharmacokinetics. Studies investigating age-related changes in the pharmacokinetics of cancer chemotherapy agents have provided conflicting evidence [2]. While some studies report age-related differences in the pharmacokinetics of cancer chemotherapy agents, most studies have reported no significant differences based on patient chronological age *per se*, or only minor differences in the pharmacokinetics of chemotherapy agents in older people. However, many of the older patients included in these studies have been selected on the basis of good performance status and

normal organ function and thus may not be representative of patients treated in routine practice. Changes observed in the pharmacokinetics of drugs in older people (when compared to younger people) result from age-related changes in physiology and organ function and/or comorbid disease [3].

Pharmacokinetic assessments in patients should incorporate consideration of factors affecting drug absorption, distribution, metabolism, and excretion.

Drug absorption can be affected by age-related changes in the physiology of the gastrointestinal tract such as decreased splanchnic circulation, reduced gastric motility and secretion, and reduction in the absorptive surface area for drugs [4, 5]. However, there are relatively few examples of drugs affected by these changes which result in clinically relevant alterations in the extent of drug absorption. Typically the rate of absorption may be reduced in older people but the extent of absorption (associated with bioavailability) remains largely unaffected [3] in older patients. Age-associated changes in gastrointestinal physiology and the potential effects on drug absorption may come into sharper focus with the trend for increased use of oral chemotherapeutic drugs. This is an important area for future investigation.

Aging is associated with changes in body composition, including a reduction in total body water, protein stores, and lean body mass with an increase in proportion of body fat [6]. Decreased intracellular water leads to a reduced volume of distribution (V) for hydrophilic drugs that primarily distribute to body water, while the relative increase in adipose tissue in older people can result in an increase in the volume of distribution of lipid-soluble drugs. This may result in a reduction in the maximum concentration achieved after a dose and prolongation of the terminal half-life of an agent in older people when compared to younger patients [7]. The reduction in serum albumin concentration in older adults results in an increase in the unbound fraction of some drugs, which may have important implications [8] for the distribution of drugs bound to albumin. Studies had shown that low serum albumin concentrations in malnourished older patients with advanced cancer resulted in a low clearance of highly albumin-bound drugs which, in turn, caused increased free drug concentration and contributed to unexpected toxicity [9].

Clearance, a measure of the efficiency of drug elimination, is the most important pharmacokinetic variable as it is the main determinant of maintenance dose rate for multiple dosing drug regimens and of drug exposure (i.e., area-under-the-concentration-time curve, AUC) after a single dose. Drug metabolism has the greatest impact on clearance for most drugs (and in turn, exposure) and is therefore likely to significantly influence a patient's beneficial and adverse response at a given dose [10]. The liver is the principal site of drug metabolism. Age-related changes in the liver include decreased liver weight, reduced hepatic blood flow, and reduced amount and activity of cytochrome P450 enzymes, in association with an overall reduction in the metabolic capacity of the liver in older people [4, 5]. Many chemotherapy agents, including the taxanes (paclitaxel and docetaxel), cyclophosphamide, and vinca alkaloid drugs (vincristine, vinblastine, and vinorelbine), and molecular targeted drugs are metabolized by cytochrome P450 enzymes, especially CYP3A4, and predominantly undergo hepatic metabolism and clearance [7]. There is clear evidence that the liver sinusoidal endothelial cells in the aging liver undergo pseudocapillarization such that the sieving function of liver is significantly reduced with age [11]. This age-related change in the liver has implications for highly protein bound drugs and large macromolecules or protein-based therapeutic agents (such as liposomal therapies, e.g., liposomal doxorubicin) with the potential for a reduction in hepatic clearance due to reduced access to the hepatocytes. Biliary excretion is a key hepatic elimination pathway for numerous drugs and their metabolites. While this is a major pathway of elimination of commonly used drugs such as the anthracyclines (doxorubicin, epirubicin, and daunorubicin), it appears to be unaffected by the age of the patient and the associated changes in physiology [12].

There is increasing recognition that inflammation associated with advanced cancer and frailty in older people has a significant effect on the regulation of drug metabolising enzymes and transporters [13, 14]. It has been demonstrated in both clinical and preclinical studies that elevated plasma concentrations of inflammatory proteins are associated with reduced hepatic drug clearance and increased toxicity from chemotherapy [15]. Inflammation has the potential to downregulate drug metabolism and transporter pathways further complicating the impact of advanced cancer and frailty on the clinical pharmacology of cancer chemotherapy agents [16–18]. Given the impact of inflammation on drug metabolism, it is, therefore, not unreasonable to hypothesize that the level of inflammatory markers in blood might correlate with variations seen in the metabolism of anticancer drugs. However, this relationship should be evaluated in prospective trials incorporating pharmacokinetic analyses of cytotoxic drugs.

Many drugs and metabolites are renally eliminated from the body. Renal clearance is the result of glomerular filtration, renal secretion (mediated by transporters), and tubular reabsorption. A reduction in glomerular filtration rate (GFR) is almost universal with age, and it has been reported that there is a decrease in the GFR of approximately 1 mL/minute for every year over the age of 40 years [19]. The kidneys' ability to appropriately concentrate or dilute urine and excrete water and electrolytes is also impaired with aging [5, 20]. This decline in renal function may be associated with an increased risk of toxicity for renally excreted cytotoxic agents and their metabolites, such as platinum compounds, alkylating agents, capecitabine, purine analogues, antimetabolites, camptothecins, and etoposide [7]. It is recommended to estimate the creatinine clearance (CrCl, as a marker of a person's renal function) with the formula of Cockcroft and Gault [21] in all patients with age of 50 years or more to guide the drug and dosage selection [3, 22, 23]. While there is now a range of equations which have been used to estimate a person's GFR based on serum creatinine concentration, the CrCL remains the best and most reliable metric to estimate renal function for the determination of drug dose regimens for renally excreted medicines [3, 21, 23].

2.1.2. Pharmacodynamics. Most of the age-related differences in drug response observed in older cancer patients are in the realm of pharmacodynamics and manifest as decrements in end-organ function [3]. Age-related changes in effector system function, organ function, and impaired homeostatic control result in age-related changes in pharmacodynamics. End-organ response is affected by physiological changes that occur with increasing age in the absence of pathology [23] or in the context of concomitant multiple pathophysiological changes. For example, the diminished homeostatic reserve in older people [3], especially those that are frail, leads to a greater impact of haematological toxicities from cancer chemotherapy agents.

The pharmacodynamic changes of aging may affect both the efficacy and the toxicity of many antineoplastic agents. The increased prevalence of MDR-1 in acute myeloid leukemia [24], increased resistance to apoptosis in follicular lymphoma [25], increased adhesion of neoplastic cells to stroma in multiple myeloma [26], decreased tumour growth fraction [27], tumour cell anoxia, and abnormal chemotherapy targets [28] are all examples of age-associated changes in pharmacodynamics that impact the efficacy of chemotherapy agents in older cancer patients.

The impaired homeostatic control associated with aging also increases the risk of short- and medium-term complications of cancer chemotherapy, including myelosuppression [29], acute cardiomyopathy [30], peripheral and central neuropathy [31] and mucositis [32], as well as long-term complications [33–36] such as chronic cardiomyopathy from anthracyclines, increased incidence of myelodysplasia (MDS), and acute myeloid leukemia (AML).

2.2. Comorbidity. The occurrence of comorbid medical conditions such as diabetes, heart disease, hypertension, arthritis, and lung disease affects many older patients [37]. The likelihood of an older person experiencing a chronic illness increases rapidly with age. In cancer patients, comorbidity limits treatment options, negatively affects treatment tolerance, and influences the presence and severity of symptoms and other complications [38]. For example, more attention should be paid to patients with ischemic heart disease when they are treated with fluoropyrimidines as a previous study has demonstrated that patients with a history of cardiac disease, particularly coronary artery disease, were significantly more susceptible to 5-FU cardiotoxicity when compared to patients without this medical history [39]. Patients with impaired lung function will face increased risk of pulmonary toxicity from bleomycin. Giving cisplatin to patients with poor cardiac function will increase the risk of the onset of heart failure due to the requirement for prehydration with large volumes of fluids. Comorbidity is also associated with poorer survival, increasing both cancer and noncancer related mortality [40, 41]. The treatment of comorbid conditions during cancer treatment may result in an increased likelihood of drug interactions. In addition, cancer treatments, including chemotherapy, radiation therapy, and other supportive care drugs, may exacerbate comorbid conditions [23].

2.3. Polypharmacy. The obvious consequence of comorbidity is the concurrent use of multiple medications. Of all the factors that are most consistently associated with adverse drug reactions, inappropriate or unnecessary polypharmacy has been considered the most important. Using multivariate analysis, studies have demonstrated that the principal contributor to adverse drug reactions in older people is inappropriate polypharmacy [23]. The harms associated with polypharmacy include increased risks of adverse drug reactions, drug interactions, increased healthcare costs, and errors in patient adherence to therapy [23]. Older age, comorbidity, recent hospitalization, female gender, depression, number of treating doctors, and practitioner characteristics are the main risk factors for polypharmacy [42]. Some medicines place older people at a significantly higher risk of adverse effects and serious drug interactions [42]. The Beers Criteria provide an important starting point to improve prescribing by limiting the use of medicines that pose a high risk of adverse effects in older people [42, 43].

3. Medical Management in Older People with Cancer

3.1. Comprehensive Geriatric Assessment. A comprehensive geriatric assessment (CGA) approach, including identification of frailty, can assist oncologists in identifying older patients who are more likely to develop severe toxicity during cancer treatment [44]. Components of the GCA, which involves a multidimensional evaluation to determine the overall health status of an older adult, have been used effectively to guide treatment planning and decision making in older adults with cancer. The CGA determines which patients may benefit from and tolerate standard cancer treatment including identifying those most likely to derive benefit from a palliative treatment approach [45].

Although the CGA is a useful tool in the assessment of older people with cancer, this approach is time-consuming and is only indicated in selected patients [46, 47]. Some clinical oncologists have used an abbreviated tool such as the VES-13 to screen patients who should go on to a full CGA [48]. In addition to this, the National Comprehensive Cancer Network (NCCN) has developed useful guidelines for pretreatment screening to determine which patients, such as those aged 70 years or more and whose hemoglobin levels are under 12 mg/dL, who may need a more comprehensive assessment [49].

3.2. Treatment Strategies. Medical oncologists have gradually started to appreciate that the approach to cancer chemotherapy that is adopted for otherwise healthy adults cannot automatically be applied to older patients with age-related changes in physiology, reduced homeostatic reserve, and comorbid medical problems. Numerous questions need to be addressed prior to commencing chemotherapy in older patients including which anticancer agents should we choose in each particular circumstance? Should we use single agents or combination chemotherapy? Should we use oral or intravenous administration? These issues must be taken into consideration by the oncologists when determining treatment strategies in older cancer patients [50]. Chemotherapy

regimens that equally optimise efficiency, tolerance, and compliance should be preferred in older cancer patients. However, the strategy of using and applying the best clinical evidence to guide treatment selection is a considerable challenge as older patients are often excluded from clinical trials.

3.3. Efficiency and Toxicity. Although standard treatment regimens may be safe and effective in older adults, the treatment of cancer in older patients requires an individualized approach. As discussed, older patients are more prone to toxicity from chemotherapeutic agents, due to age-related changes in both pharmacokinetics and pharmacodynamics [51]. The common treatment toxicities of particular concern when caring for older adults with cancer are myelosuppression, mucositis, cardiotoxicity, neurotoxicity, and musculoskeletal side effects [52].

4. Frailty

Frail older people are defined as those with an excess reduction in lean body mass and mobility, poor tolerance to therapy and fatigue, presence of geriatric syndromes, and/or the presence of multiple comorbidities [53]. Frailty reflects a common view of aging, indicating a critically exhausted functional reserve [54]. The recognition of frailty is particularly useful in planning cancer chemotherapy in older individuals. It may allow the practitioner to identify patients who are most at risk of chemotherapy-induced adverse outcomes and to implement a strategy to monitor and prevent these complications [53]. The frail older cancer patient needs a different approach to chemotherapy prescription. Sarcopenia, inflammation, and poor nutritional status are associated with frailty in older people [52, 54, 55]. The factors have the potential to significantly affect the clinical pharmacology of cancer chemotherapy agents. Simple pharmacological palliation of symptoms, rather than administration of potentially toxic chemotherapy, may be the preferred option in frail older people; however, the rationale for this option may need careful, sympathetic, and lengthy explanation to the patient and their relatives. Relatively less toxic single agent therapies may be the best choice in many solid tumour types, but may not be effective in rapidly growing tumours such as high-grade non-Hodgkin lymphomas or acute myeloid leukaemia [50].

5. Summary and Outlook for the Future

Older people represent an increasing proportion of the population and are a heterogeneous patient group at high risk for developing cancer. Age-related physiological changes may have a considerable impact on the pharmacokinetic and pharmacodynamic properties of cancer chemotherapy agents. For anticancer drugs, which have a low therapeutic index even in optimal circumstances, pharmacological changes can result in dramatic consequences, such as excessive increases in drug concentrations that produce severe and even life-threatening toxicities when standard dosing regimens are employed. In general, chronological age *per se* is not a contraindication to receiving cancer chemotherapy [56]. Comorbidity and poor functional status, which may be present in a significant number of the older patient population, are the main limiting factors. The key strategy is to focus on the clinical pharmacology of cancer chemotherapy agents and to individualises treatments which can be achieved by understanding the nature and extent of age-related changes in physiology and organ function. Older cancer patients, especially those with comorbidities, have been historically omitted from clinical trials resulting in study populations that are selected for their fitness and thus not representative of typical older cancer patients. It still remains a challenge to tailor and deliver the most beneficial treatments for those over the age of 65 years, taking into account comorbidities and physiologic reserves. Fortunately, there are clinical trials within various cooperative groups directed toward the development of effective and safe treatment strategies for the older people with cancer. These considerations will have important implications for training the next generation of healthcare professionals to meet the future needs [57] of older people living with cancer.

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Review Article

Age-Related Changes in the Hepatic Pharmacology and Toxicology of Paracetamol

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Optimal pharmacotherapy is determined when the pharmacokinetics and pharmacodynamics of the drug are understood. However, the age-related changes in pharmacokinetics and pharmacodynamics, as well as the increased interindividual variation mean optimal dose selection are a challenge for prescribing in older adults. Poor understanding of how hepatic clearance and toxicity are different with age results in suboptimal dose selection, poor efficacy, and/or increased toxicity. Of particular concern is the analgesic paracetamol which has been in use for more than 50 years and is consumed by a large proportion of older adults. Paracetamol is considered to be a relatively safe drug; however, caution must be taken because of its potential for toxicity. Paracetamol-induced liver injury from accidental overdose accounts for up to 55% of cases in older adults. Better understanding of how age affects the hepatic clearance and toxicity of drugs will contribute to evidence-based prescribing for older people, leading to fewer adverse drug reactions without loss of benefit.

1. Introduction

Paracetamol remains one of the most studied agents that cause hepatotoxicity due to its clinical relevance and to its dose-dependent hepatotoxicity in animals and humans [1]. Paracetamol is an effective analgesic agent and represents the first-line analgesic therapy for nonmalignant pain [2]. However, the use of paracetamol is limited by its potential to cause hepatotoxicity. With old age, there is an increase in disease for which medications may provide benefit; however, the incidence of serious adverse drug reactions (ADRs) also increases with increasing age, even after controlling for increased medication use [3]. In older adults, most ADRs, including drug-induced liver injury (DILI), are dose-related [4]. Therefore, optimising the safety and efficacy of medication use in older adults is important.

For most drugs, the evidence base for dose adjustment in older people is limited to pharmacokinetic studies in small populations of healthy volunteers. There is very little data available on the clinical outcomes of dose adjustment, particularly in the frail aged. In all age groups, an important susceptibility factor for hepatotoxicity is genetic variability [5]. In older people, this may be compounded by the multi-factorial large interindividual variation in response to medications further increasing the risks of toxicity and poor efficacy [5]. This is a particular concern in frailty, a condition of increased vulnerability to adverse events [6]. Although monitoring for clinical response is essential to optimise efficacy and reduce toxicity, the detection of adverse effects of medications in older patients may be complicated by nonspecific presentation as "geriatric syndromes" [7]. In addition, age-related changes in the pharmacokinetics and

pharmacodynamics of drugs further compounds the risk of toxicity. In older adults, the clinical increased risk of paracetamol hepatotoxicity is likely to be related to dosing that does not account for decreased liver volume with age, and to frailty and malnutrition [8]. However, recent discoveries about the ageing liver identify novel mechanisms for age-related changes in hepatic pharmacology and toxicology (summarised in Figure 1). In this paper, we describe the age-related physiologicalchanges, with particular attention to the liver specific changes, and how they can impact on hepatic pharmacology and toxicology of paracetamol.

2. Physiological Changes in Ageing

The most marked pharmacologic change with ageing is increased interindividual variation. The most significant pharmacokinetic change in ageing is related to the decreased hepatic mass, uptake, and blood flow [9, 10] and to decreased renal function [11], impairing the clearance of many drugs and their metabolites [12]. Table 1 summarises the physiological changes associated with ageing and frailty that can impact on the pharmacokinetics and pharmacodynamics of drugs.

Changes in the pharmacodynamics of drugs in old age is related to changes in drug receptors, physiologic reserve and in response to injury [12]. However, these changes have not been as well characterised in ageing as the pharmacokinetic changes. The cardiovascular and central nervous systems are the two best described. A reduction in the responsiveness of the cardiac and β-adrenergic system has been observed in older adults [14]. In the central nervous system, the numbers of dopaminergic neurons and dopamine D2 receptors decrease with age resulting in extrapyramidal side effects [26]. Studies in animal models suggest that increased sensitivity to narcotic and anaesthetic agents may be due to alteration of opioid receptors (decreased μ-opioid receptor density and increased affinity) in old age [15].

Ageing is associated with changes in body composition, including a reduction in total and lean body mass, and a relative increase in fat mass [12, 27], which may affect the volume of distribution and loading dose of drugs. Sarcopenia, defined as loss of muscle mass and strength with ageing, increases with age and is associated with frailty [5, 27]. It can be the result of concomitant diseases, neuroendocrine dysregulation and/or chronic inflammation [27].

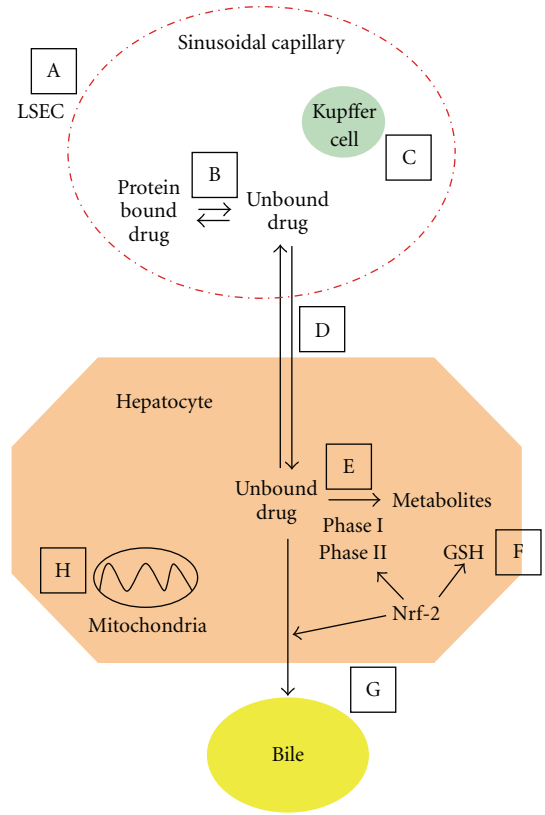

Figure 1: Hepatic pharmacology and toxicology in old age. (A) Pseudocapillarisation (thickening, defenestration, and basement membrane formation) of the liver sinusoidal endothelial cells (LSECs) may affect susceptibility to drug-induced liver injury (DILI); (B) Changes in protein binding in old age affect the amount of free drug available for clearance; (C) Dysregulation of Kupffer cell activation may alter inflammatory response to DILI; (D) Pseudocapillarisation of the LSECs, and any changes in transporters, may alter drug transfer from the blood to hepatocytes; (E) Age-related changes in hepatic metabolism affect drug clearance: phase I metabolism is reduced, and changes in phase II metabolism are less well understood; (F) Reduced glutathione (GSH) in old age increases injury by toxic metabolites; (G) Expression of hepatic transporters in response to drug toxicity is poorly described in old age and affects biliary excretion of drugs and their metabolites; (H) Changes in mitochondrial structure and function in old age alter response to reactive oxygen species and cell death pathways. Steps (E), (F), and (G) are regulated by nuclear factor E2-related factor 2 (Nrf-2) which has reduced hepatic expression in old age. Figure adapted from [24, 25].

3. Liver Specific Changes in Ageing

The age-related reduction in liver size is noted to be in the order of 25 to 35%, which has been confirmed in many species including humans [9, 28–30]. The main age-related change in the physiology of the liver is a substantial reduction in blood flow of about 40% [29] which has been postulated to be due to leukocyte accumulation in the sinusoids and narrowing of sinusoidal lumens due to pseudocapillarisation and dysfunction of the liver sinusoidal endothelial cells (LSECs) [31].

3.1. Pseudocapillarisation of the Liver Sinusoidal Endothelium. The sinusoidal endothelium is a thin fenestrated endothelium lacking a basal lamina and punctuated with fenestrations of 50 to 200 nm in diameter grouped together in clusters known as liver sieve plates [32–35]. With ageing, the LSECs undergo ultrastructural changes termed pseudocapillarisation [16] that include that include loss of fenestrations, thickening of the endothelium, perisinusoidal collagen deposition, and basal lamina formation [16, 36, 37], which may affect hepatic drug disposition. Recently, the endocytic capacity of LSECs was reported to be reduced in old

Table 1: Physiological changes associated with ageing and frailty that can impact on the pharmacokinetics and pharmacodynamics of drugs.

Physiological change	Pharmacokinetic consequences
↑ Gastric pH ↓ Secretory capacity ↓ Gastrointestinal blood flow ↓ Absorption surface ↓ Gastrointestinal motility	*Delay* in absorption no change in the overall *extent*
↑ Body fat	↑ Vd and $t_{1/2}$
↓ Lean body mass ↓ Total body water ↓ Serum albumin ↑ α1-acid glycoprotein	↑ Plasma concentration and ↓ Vd of hydrophilic drugs ↑ Free fraction of highly protein-bound acidic drugs ↓ Free fraction of basic drugs
↓ Hepatic blood flow ↓ Hepatic mass ↓ CYP content	↓ First-pass metabolism Phase I metabolism of some drugs may be slightly impaired ↓ / ↔ Phase II in fit older adults, ↓in frail ?/ ↓ Phase III
Pseudocapillarisation of the liver sinusoidal endothelium	Impaired transfer of chylomicrons and possibly medications from sinusoid to space of Disse
↓ Renal blood flow and glomerular filtration rate ↓ Tubular secretion	Renal elimination of drugs can be impaired altering drug half-life
Physiological change	**Pharmacodynamic consequences**
↓ Blood supply to brain ↓ Baroreceptor activity	↑ Sensitivity to centrally acting drugs such as benzodiazepines
↓ Resting heart rate, stroke volume, and cardiac output	↓ Response to beta blockers such as metoprolol
↓ Plasma renin ↓ Urine aldosterone	
↓ Hepatic GSH Dysregulation of Kupffer cells Dysregulation of the immune system Mitochondrial dysregulation	↓ Detoxification ability of the liver Dysregulation of immune response to drugs and other toxins ↑ Susceptibility to DILI

↓, decreased; ↑, increased; ↔, no change; ?, unknown; CYP, Cytochrome P450; Vd, volume of distribution; $t_{1/2}$, half-life; DILI, drug-induced liver injury; GSH, glutathione; adapted from [13] and references [2, 11–23].

age, which may be especially important in situations with increased circulatory waste loads [38].

3.2. Dysregulation of Kupffer Cell Activation. Kupffer cells (KCs) are the resident phagocytic macrophages in the liver, which represent the largest population of fixed macrophages in the body and account for approximately 20% of nonparenchymal cells in the liver [39]. KCs have diverse functions, including phagocytosis, endocytosis, immuno-modulation, and synthesis and secretion of numerous biologically active mediators [39, 40]. Furthermore, the dual role of activated KCs in releasing both pro- and anti-flammatory mediators during the different stages of liver injury and regeneration has been demonstrated [39]. It is likely that dysregulation of the KCs with ageing may alter the inflammatory response to DILI; however, there is conflicting evidence. For example, while there is a basal increase in the numbers of KCs in old rats [40], their activation in response to toxic doses of cadmium and endotoxin is decreased [41, 42]. However, KCs in lipopolysaccharide-treated had showed no difference in activity with the phagocytosis of fluorescent beads being similar across age groups [43]. The role of KCs in paracetamol-induced hepatotoxicity will be discussed below.

3.3. Age-Related Changes in Hepatic Metabolism Affect Drug Clearance. Age-related changes in hepatic metabolism will affect drug clearance and toxicity. In general, there is a reduction in Phase I metabolism in vivo with normal ageing

Table 2: Changes in the cytochrome P450 activity with ageing.

CYP Enzyme	Change with ageing	Probe drug used	Confounding factors
CYP1	↓	Theophylline	Ethnic polymorphisms, sex differences, lifestyle, and disease
CYP2 CYP2C9 CYP2C19	↓ (∼25%)	Phenytoin Warfarin Omeprazole	Age-related effects, and unrecognised environmental effects, and pharmacogenetic variation
CYP2D6	↓ Older women ↓ Older Japanese men	Dextromethorphan Haloperidol	Genetic polymorphisms
CYP2E1	↓ Aged rats ↔ Aged mice ↓ Activity in human liver microsomes ↔ Human hepatocytes	Chlorzoxazone	? Gender-conflicting results Polymorphisms
CYP3A CYP3A4	↓ Aged rodents ↔ Humans ↑ CL in women, no age effects	Cyclosporine, Erythromycin, Verapamil, Midazolam	Inducers Inducers
Multiple	↓	Antipyrine	Metabolised by CYP3A4, 1A2 and 2C8/9

CYP, cytochrome P450; ↓, decreased; ↑, increased; ↔, no change; ?, unknown; CL, clearance; adapted from [13, 52–55].

in the order of 30%–50% [24]. Phase II metabolism appears to be maintained in the healthy elderly but reduced in the frail [24]. The Phase III transporters have not been well described in humans.

The Phase I drug metabolising enzymes (DMEs) consist of the superfamily of CYP450 enzymes. Animal studies have indicated that total CYP content is reduced in aged rats [44]. In humans, it has been suggested that CYP450 content declines at a rate of 0.07 nmol/g of liver after 40 years of age [45]. However, there is conflicting evidence on the CYP activity with age (Table 2). For example evidence from liver biopsies of surgical patients indicate that normal ageing does not affect the activity of human CYP2E1, other evidence suggests that CYP2E1 activity may decrease with age [46]. Furthermore it has been suggested that the induction of CYP2E1 could also be affected by advanced age [47]. Interestingly it has shown that sex and concurrent medications have a greater effect than chronological age on CYP3A substrates in older patient populations [48, 49]. Frail older persons do not have slower erythromycin breath test results compared with non frail older persons potentially indicating preserved CYP3A activity as well as P-glycoprotein (P-gp) transport, in frailty [48].

However, it can be assumed that there is some minor impairment in CYP activity with ageing given that there is a small decline (in the order of 20% observed in patients aged 25–75 years) in antipyrine clearance with age and antipyrine is metabolised by multiple CYPs [45, 50, 51]. The effect of disease, concurrent medications and frailty, and comorbidity on CYP activity still needs to be investigated given that these studies were conducted in relatively healthy volunteers.

Phase II metabolism acts to increase hydrophilicity of the compounds, and thereby enhance excretion in bile and or urine [63]. Enzymes include the sulfotransferases, UDP-glucuronosyltransferases (UGTs), and glutathione s-transferases (GSTs). Most data suggest that the Phase II conjugation pathways are not altered by ageing [13, 52]. However, a recent reanalysis of pharmacokinetic studies in old age found that the apparent preservation of Phase II metabolism may have been confounded by inadequate consideration of protein binding [64]. Therefore, suggesting that Phase II metabolism is impaired in healthy ageing [64]. This is consistent with animal studies in which transcript profiles for the glucuronidation, sulfation, and glutathione conjugation genes are reportedly decreased in aged Fischer 344 rats [65].

The Phase III hepatic pathway encompasses the transporters on the basolateral and apical sides of the hepatocytes, which function to remove xenobiotics from the portal blood and to excrete them or their metabolites in to bile or blood, and includes the bile transporter P-gp [66–68]. The expression of hepatic transporters in response to drug toxicity is poorly described in old age and will affect biliary excretion of drugs and their metabolites. There are few human studies on Phase III hepatic metabolism in ageing. P-gp expression is increased in aged Fischer-344 male, but this is specific to the liver [69]. A small study of healthy volunteers found decreased P-gp activity in the blood brain barrier of five older healthy volunteers, age range 59–68 years [70]. It must be noted that the wide genetic interindividual variation in expression of P-gp [71] may be further be confounded by the increasing heterogeneity with age [67].

Ageing has been associated with decreased mRNA expression of organic anion-tran sporting polypeptide (Oatps) including Oatp1a1, Oatp1b2, and Oatp2b1, as well as organic cation transporters (Octs) Oct1, and sodium/taurocholate-cotransporting polypeptide in aged mice [53]. Furthermore, the mRNA expression of several efflux transporters including Multidrug resistant protein (Mrp)-2, Mrp6 and Mrp3 have been shown to be significantly reduced in old age in mouse livers [53]. However, what this means in terms of activity and how it translates to humans still needs to be determined.

3.4. Mitochondrial Structure and Function in Old Age. Changes in mitochondrial structure and function in old age [72] alter the response to reactive oxygen species and cell death pathways. It appears that malfunction and decrease of biogenesis of mitochondria seem to exert some of the most potent effects on the organism [72]; however, the exact mechanism still needs to be elucidated.

3.5. Glutathione in Old Age. Glutathione (GSH) has several important functions including detoxification of electrophiles, maintenance of essential thiol status of proteins and other molecules, scavenging of reactive oxygen species (ROS), providing cysteine as well as modulation of critical cellular processes such as DNA synthesis, microtubular-related processes, and immune function [73, 74]. These reactions are catalysed by the GSTs [74]. Hepatic GSH is decreased in aged rats [75, 76] and in aged mice [77, 78]. Serum GSH and the levels of its associated enzymes are decreased in ageing in humans [79]. In rats, this age-related decrease has been shown to be further exacerbated by ethanol consumption [80] which may be a problem in chronic alcoholics.

3.6. Nrf-2 in Old Age. Nuclear factor E2-related factor 2 (Nrf-2) regulates the transcription of antioxidant genes including genes for the Phase II conjugation enzymes (e.g., UGTs), glutathione homeostasis, stress response, and transporter proteins through the antioxidant-responsive element. Nrf-2 downregulation with ageing has been suggested as one mechanistic explanation for reduced Phase II metabolism in old age [81].

4. Implications of the Age-Related Alterations in Hepatic Pharmacology and Toxicology

A decline in liver volume and liver blood flow with ageing may be a major component of age-related alterations in the liver, leading to the fall in clearance of many of the drugs whose pharmacokinetics have been found to be altered with age [9]. Hepatic clearance is influenced by substrate delivery to the liver parenchymal cells and by the inherent metabolic capacity of the hepatocytes. Therefore, any change in the LSECs, including pseudocapillarisation, may alter drug transfer from the blood to hepatocytes. Age-related pseudocapillarisation has been shown to be associated with impaired transfer of lipoproteins as well as with paracetamol across the fenestrations [82, 83] as illustrated in Figure 2.

Changes in the inherent ability of the liver to detoxify toxic metabolites will lead to increased susceptibility to DILI. This may be due to the age-related dysfunction and reduced biogenesis of mitochondria [72], and/or the age-related reduction in Phase II metabolism and reduced hepatic GSH in old age [84], secondary to reduced transcriptional activity of Nrf-2 [81].

Interestingly "the mitochondrial hypothesis" implies that the gradual accumulation of initially silent mitochondrial injury which, when a critical threshold is reached, abruptly triggers liver injury [85]. This could explain why DILI does not affect all individuals equally (duration of exposure is not the same for all individuals), the delay in developing DILI by weeks or months (accumulation of deficits to reach a threshold), and why increasing age is a risk factor (due to duration of exposure or mitochondrial changes in ageing) [86]. The role of these changes and their effect on paracetamol-induced liver injury will be discussed below.

5. Paracetamol-Induced Liver Injuryin Ageing

Paracetamol (or acetaminophen) is a p-aminophenol derivative which was discovered at the John Hopkins University in 1877 [87]. Due to its safety profile, paracetamol is particularly useful in older adults; however, caution must be taken because of its potential for toxicity [88]. Paracetamol has the potential to cause liver damage and even liver failure in overdose and now case reports are emerging of people developing significantly increased ALT concentrations following therapeutic dosing [89], even in the absence of risk factors [89, 90]. Paracetamol causes dose-dependent hepatotoxicity through the metabolic bioactivation of the parent drug to toxic metabolite [1].

5.1. Epidemiology. A recent systematic investigation by the WHO Collaborating Centre for International Drug Monitoring reported that since 1969, paracetamol has been one of the five most common drugs associated with fatalities [91]. After 1990, there was a shift from halothane (immunoallergic DILI) being the most common drug associated with a fatal outcome to paracetamol (dose-dependent DILI) [91, 92]. Table 3 shows selected reports of paracetamol-induced hepatotoxicity deaths and transplants, with special reference to those aged >60 years, in the United Kingdom, United States, Canada, Malaysia, and Australia for the period 1989–2010. In the United States, paracetamol is responsible for approximately half of the cases of acute liver failure [1].

5.2. Hepatotoxicity. At therapeutic doses paracetamol metabolised primarily in the liver to nontoxic metabolites via Phase II metabolism (conjugation) with glucuronide and sulphate, or cysteine [93]. A small amount of drug undergoes Phase I CYP450-mediated N-hydroxylation to form N-acetyl-p-amino-benzoquinone immine (NAPQI), a toxic metabolite [93–95]. The most important isoform responsible for this CYP450-mediated metabolism is CYP2E1, but

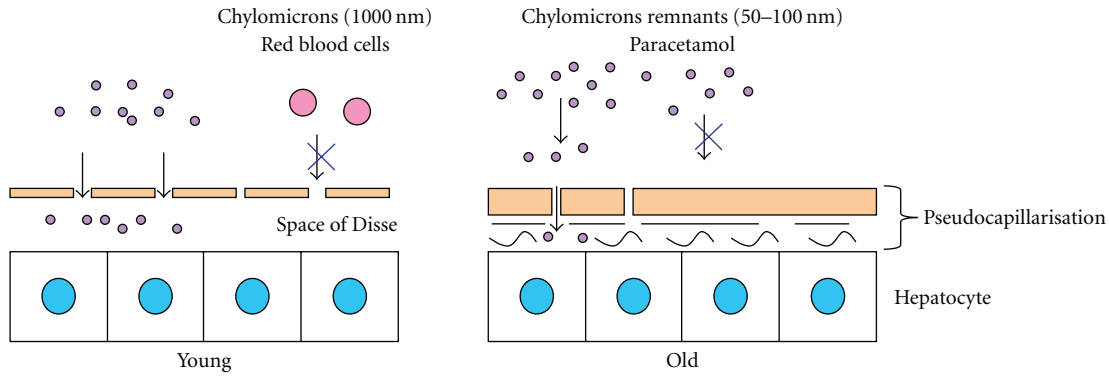

Figure 2: Age-related pseudocapillarisation of the liver sinusoid impairs the transfer of lipids (chylomicrons remnants) and paracetamol across the fenestrated liver sinusoidal endothelial cells (LSECs). Adapted from Le Couteur et al., 2002 (20).

Table 3: Selected reports of paracetamol-related hepatotoxicity, deaths, and transplantsin the United States, Canada, United Kingdom, Malaysia, and Australia for the period 1989–2010. Only studies that have included a sub grouping for "older adults", defined as those aged > 60 years, are included.

Source	Approximate population Size	Cases/million population/year	% of Reports for those aged> 60 years	% of Reports unintentional	Reference
Spontaneous ADR reports, AUS 1990–2010	17–22.5 million	0.04 deaths	37.5% deaths	NR	Pers. Comm. Graeme Harris, ACSOM, 24/8/2010
Ballarat Hospital Records, AUS 2000–2003	0.2 million	240 hospitalisations	2.6% hospitalisations	4.7%	[56]
Penang General Hospital, Malaysia 2000–2002	Approx 1.3 million	42.3 cases of poisoning	1.2% of poisoning cases	33.3%	[57]
Calgary, Canada1995–2004	1.1 million	140.2 hospitalisations	4.5% hospitalisations	13%	[58]
US Transplant Centres1998–2001	17 tertiary care centres	NR	6% ALFs 6.8% deaths	57% ALFs	[59]
US 1990–2001	250 million	1.83 deaths	4% hospitalisations 14% deaths	23 % hospitalisations 22% deaths	[60]
Cardiff, UK 1989–2002	Approx 2.9 million	185 hospital admissions	1.6 % of admissions in adults 60–69 years 1.8% of admissions in adults >70 years	All intentional	[61]
England and Wales 1993–1998	NR	15720 deaths, 13% due to paracetamol alone, 5.8% due to paracetamol and other drugs	11.5% deaths per million males during 1993–1998 14.2% deaths per million females during 1993–1998	NR	[62]

NR, not reported; US, United States; UK, United Kingdom; AUS, Australia; ADR, adverse drug reaction; ACSOM, Advisory Committee on the Safety of Medicines; ALF, acute liver failure; APAP, paracetamol.

CYP3A4 and CYP1A2 are also involved [93]. Under normal circumstances, NAPQI combines with sulphydryl groups in hepatic glutathione and is neutralized [93, 96]. The major conjugates, glucuronide and sulfate being more water-soluble than the parent drug, are both eliminated from the liver and blood mainly via the urine, with a small amount of the glucuronide conjugate eliminated via the bile [93, 97, 98].

Following ingestion of large amounts of paracetamol, conjugation pathways become saturated resulting in increased use of the CYP450 pathway, increased NAPQI formation and increased depletion of hepatic glutathione [97, 99]. The direct mechanism of paracetamol-induced liver injury involves the formation of the toxic metabolite, NAPQI, from paracetamol by the enzymes of the liver [100].

NAPQI can directly interact with macromolecules in the cell causing protein dysfunction, lipid peroxidation, damage of DNA, and oxidative stress [100]. Dysfunction of mitochondria may also result thereby in interrupting energy production and disrupting ionic gradients and intracellular calcium stores to result in cell death and liver damage [100, 101].

The formation of reactive metabolites such as NAPQI is an important initiating factor for DILI. It is the inflammatory immune response and the balance between the protective and toxic signalling processes of the cells involved in this response that determines the severity and progression of liver injury [102]. Holt and Ju (2006) suggest that hepatocyte stress or death, as a result of the reactive metabolite induced damage, causes the release of signals that stimulate activation of the innate immune cells of the liver [100]. KCs, natural killer cells and neutrophils, are part of this response [103] and are recruited and activated. These cells produce proinflammatory cytokines and mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and interferon (IFN)-γ [104–106]. Other mediators released by these immune cells are protective and anti-inflammatory such as IL-10 [106] and IL-6 [107]. However, there is much disagreement between the studies, and the exact role of each of the cell types and mediators in DILI generally, as well as in paracetamol-induced liver injury, has yet to be fully determined [100].

5.3. The Role of Kupffer Cells in Paracetamol Induced Hepatotoxicity. Paracetamol-induced hepatotoxicity has been attributed in part to activation of KCs secondary to hepatocyte damage initiated by NAPQI [108, 109]. It is believed that Kupffer cell activation results in the release of a wide range of proinflammatory mediators capable of causing further hepatic injury [110]. However, there is controversial evidence surrounding the role of KCs in paracetamol-induced hepatotoxicity. The numbers of these F4/80 positive cells in the liver are increased following paracetamol treatment [111, 112]. Yet, macrophage depletion has been shown to have a role in both the protection [113] and potentiation of liver injury [114]. Pretreatment of rats with macrophage inactivators, such as gadolinium chloride and dextran sulfate, has been shown to decrease hepatic injury from paracetamol in rats [110, 115]. This was also observed in a mouse model of hepatotoxicity [113, 114] with the protection being ascribed to decreased formation of reactive oxygen and nitrogen species [113]. The use of liposome-encapsulated clodronate to deplete KCs from the liver [116] revealed a hepatoprotective role in a mouse model of paracetamol-induced liver injury [114]. Furthermore, the significant decrease in the levels of several cytokines and mediators, including IL-6-, IL-10-, and IL-18-binding protein may suggest that KCs mediate their beneficial role via the release of such soluble factors [114]. In support of this, it was recently suggested that a disturbance in the T-helper (Th)-1/Th-2 cytokine balance could play an important role in the pathogenesis of paracetamol-induced liver injury [117].

5.4. The Role of LSECS and Microvasculature Disturbance in Paracetamol Hepatotoxicity. It was recently suggested that the hepatoprotective role of KCs may be mediated, in part, via regulation of LSEC homeostasis and integrity [118]. In mice, the early events occurring in the hepatic microvasculature following paracetamol treatment include LSEC injury, which was exhibited by the swelling of LSECs, and the penetration of erythrocytes into the extra sinusoidal space [119]. Interestingly, these findings precede hepatocyte injury and suggest that the LSECs are a direct and early target during paracetamol hepatotoxicity [119, 120]. Furthermore, the structural and functional changes in LSECs could contribute to the initiation or progression of paracetamol-induced liver injury [119]. Taken together, this indicates that reduced sinusoidal perfusion and increased Kupffer cell activity participate in the development of liver injury elicited by paracetamol [119].

5.5. The Effect of Ageing on Susceptibility to Paracetamol-Induced Liver Injury. Risk factors for paracetamol hepatotoxicity include malnutrition which results in depletion of glutathione [121], chronic alcohol consumption, which acts to both reduce glutathione stores and induce CYP2E1 [122], and concurrent use of CYP-inducing drugs such as Isoniazid [123]. Inflammation as a result of bacterial or viral infection has also been identified as a risk factor for paracetamol hepatotoxicity [124] along with liver disease [125]. Interestingly, polymorphisms in the CYP2E1 enzyme causing altered acetylation status have been shown to be a factor influencing isoniazid hepatotoxicity [126, 127]. Conceivably, this may also be applicable to paracetamol with those individuals with a "rapid acetylator" phenotype may have accelerated production of the hepatotoxic NAPQI, however this needs to be substantiated further. Furthermore, the effect of age on these risk factors is not fully understood. Figure 3 describes the risk factors for paracetamol hepatotoxicity and the effect of age as a modifier of the risk factor. Increasing age is associated with increased time to presentation [128], resulting in poorer outcome. Interestingly, in rats the risk of hepatotoxicity from paracetamol decreases with increasing age [129]. It also must be acknowledged that elevated liver enzymes after exposure to paracetamol have occurred in adults who have none of the reported risk factors for paracetamol toxicity [89, 130].

The risk of hepatotoxicity from therapeutic doses of paracetamol in older people is not well defined. Older frail hospital in patients taking therapeutic paracetamol for five days do not have an increased risk of raised ALT compared to younger patient, although the clinical implications of such findings are not clear [131]. In people aged $\geq$ 65 years, the clinical increased risk of paracetamol hepatotoxicity is likely related to dosing that does not account for decreased liver volume with age, and to frailty and malnutrition [8]. Ageing and frailty are associated with a loss of reserves and increased state of vulnerability [132]. Therefore it is likely that the older frail patient will be at increased risk of DILI from therapeutic doses of medications. Changes in drug clearance in old age affect the formation and clearance of the toxic metabolites and therefore the susceptibility to DILI [67]. Interestingly one determinant of the variability in susceptibility to hepatotoxicity appears to be inflammatory

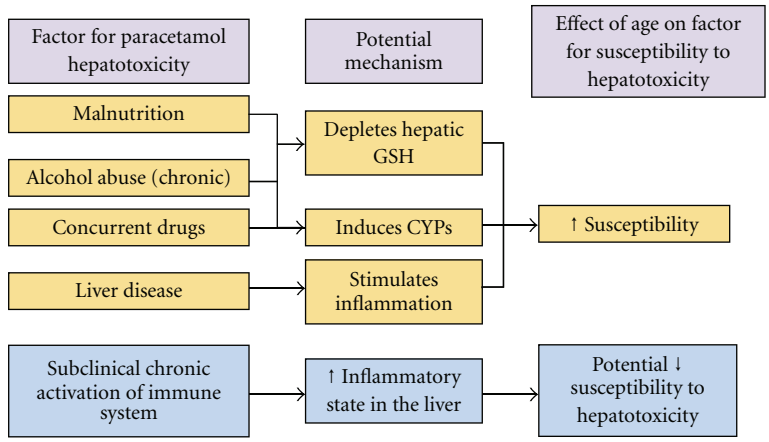

Figure 3: The effect of age on risk factors for paracetamol-induced hepatotoxicity and the potential mechanism through which they may act.

stress [1]. Subclinical chronic activation of the immune system in older people [133] is likely to result in decreased response to injury. Figure 4 summarises the paracetamol hepatotoxicity pathway and identifies potential parts of the pathway at which ageing may act to increase or decrease the susceptibility to toxicity. However, this is likely to vary between individuals.

5.6. Detection and Management of Paracetamol-Induced Liver Injury. Clinically, paracetamol overdose is associated with three main stages. The first lasts for approximately 24 hours and involves nonspecific gastrointestinal symptoms such as nausea, vomiting and abdominal pain with minimal elevation in serum liver enzyme concentrations. The second stage, from 24–72 hours, involves most notably the elevation of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations released from damaged hepatocytes [134–136]. Serum ALT and total bilirubin are the most common biomarkers used to detect and manage hepatocellular injury [137]. Serum ALT is more liver specific than AST and is a very sensitive detector of hepatocellular necrosis; however, it cannot distinguish DILI from necrosis resulting from other causes such as viral hepatitis, alcohol consumption, or other unexplainable reasons [137–139]. In patients with *a priori* elevated transaminases, this is further complicated due to the lack of guidelines as to what contributes a significant increase [138]. In older people, reduced liver size may mean transaminases do not increase as substantially as for younger people [29, 140].

Serum paracetamol concentrations are used to guide treatment in overdose [142]. However, there is still limited evidence on the relationship between therapeutic serum paracetamol concentrations and risk of hepatotoxicity, as in older adults high serum concentrations are not necessarily associated with increased ALT levels [131]. The third stage of clinical paracetamol overdose develops in the next 24 hours, with the symptoms and outcome varying from full recovery to death depending on the severity of the liver damage [101]. Liver biopsy reveals a centrilobularnecrosis, with periportal sparing and little or no inflammatory reaction [93, 134]. In severe cases, acute renal failure may occur [93]. Pharmacometabolonomics may help predict individuals at risk of paracetamol hepatotoxicity in the future [143].

5.7. Management and Treatment of Paracetamol-Induced Liver Injury. Early intervention is essential, as the aim of treatment is to prevent progression to acute liver failure. Paracetamol remains the only hepatotoxin to have effective pharmacotherapy, N-acetylcysteine (NAC), based on well-established nomograms [142]. The benefit of NAC extends those who have developed fulminant hepatic failure [144]. In older people, increased age is associated with increased time to presentation which may be explained in part by the higher proportion of accidental overdose in older patients [128]. By the time older adults present, NAC may no longer be beneficial despite being indicated for late presenters (10–24 hours after overdose) [144].

Adjunctive therapy such as corticosteroids or ursodeoxycholic acid is based on anecdotal evidence. The pharmacotherapy of end-stage liver disease (diuretics, beta-blockers) is the same as for other causes of liver disease [144]; however, this is not well described in ageing [86]. Older people do however suffer more ADRs to beta blockers and diuretics [145]. Studies in mice have indicated the usefulness of cimetidine both alone (two doses at 2 and 6hours post paracetamol) and in combination therapy with NAC to reduce ALT/AST concentrations and increase hepatic GSH following paracetamol overdose [146]. Cimetidine may have limited use in hospitalised overdose patients with no effect on ALT/AST being observed if the cimetidine was given after 8 hours after overdose [147]. Additionally, cimetidine has anticholinergic side effects in older adults, which are well known to be associated with poorer functional outcomes [148, 149], further limiting the use of this adjuvant in older patients in clinical care.

6. Future Directions

A number of antioxidants have shown promise in protecting against paracetamol-inducedliver injury. These include

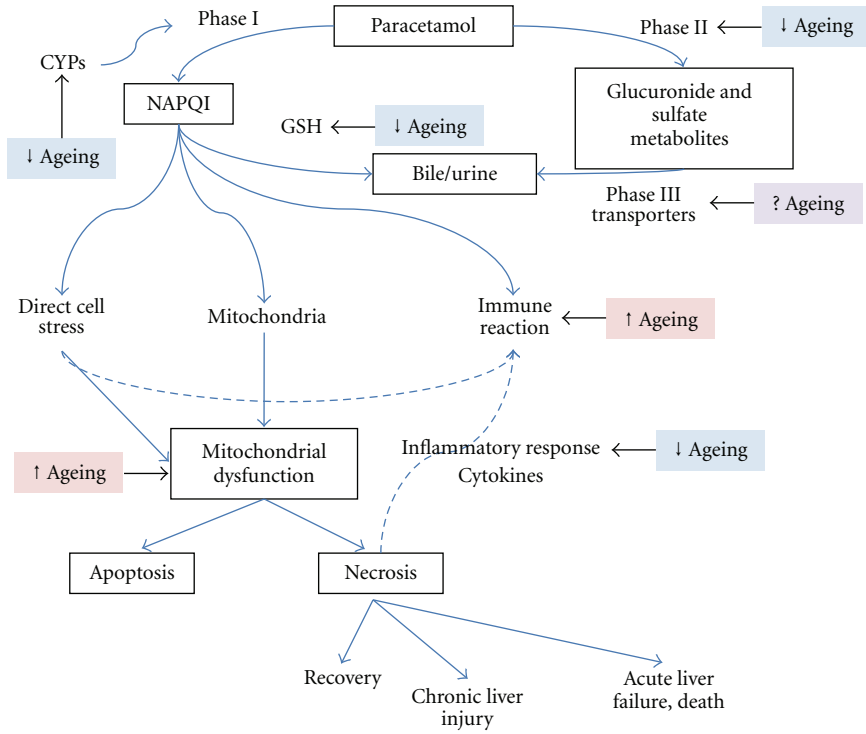

Figure 4: The effect of age on the hepatotoxic pathway for paracetamol-induced liver injury. At therapeutic doses, paracetamol metabolised primarily in the livervia the Phase II metabolism (conjugation). A small amount of drug undergoes Phase I CYP450-(CYP-)mediated N-hydroxylation to form N-acetyl-p-amino-benzoquinone immine (NAPQI), a toxic metabolite which is conjugated with hepatic glutathione (GSH) and is neutralised. The major metabolites are excreted via the urine or bile by Phase III transporters. Saturation of conjugation pathways results in increased use of the CYP450 pathway, increased NAPQI formation, and increased depletion of hepatic glutathione. NAPQI can cause injury through direct cell stress, direct mitochondrial inhibition, or through immune reactions. Initial injury leads to mitochondrial dysfunction leading to either apoptosis of damaged cells, or necrosis with recovery, chronic liver injury or actual liver failure, and death as potential outcomes. Additionally, necrosis can stimulate the inflammatory response leading to cytokine release and further potentiation of the immune reaction. Ageing can act at multiple parts of the pathway to either increase (↑) or decrease (↓) susceptibility to hepatotoxicity. It must be noted, however, that this is likely to vary between individuals. The effect of ageing on Phase III transporters is somewhat unknown (?) in humans. Picture adapted from Russmann et.al., 2009 [141].

silymarin [150], resveratrol [151], Ukrain [152], *Garcinia kola* seed extract [153], *Ginkgo biloba* extract [154], L-carnitine [155] and oleanic acid [156]. All propose to protect from hepatotoxicity through reduction of oxidative stress mechanisms. However, it must be noted that while these compounds have shown promise in the laboratory setting in animal models, they all suffer the limitation of being given prior to paracetamol overdose. Interestingly, prostagland E2 given either before or 2 hours after paracetamol overdose showed significant hepatoprotective effects in mice [157]. However, their efficacy as a therapy postparacetamol treatment and in humans in the clinical setting still needs to be substantiated.

7. Conclusion

Optimal pharmacotherapy is determined when the pharmacokinetics and pharmacodynamics of the drug are understood. However, the age-related changes in pharmacokinetics and pharmacodynamics aswell as the increased interindividual variation mean optimal dose selection is a challenge for prescribing in older adults. Paracetamol remains the first-line analgesic of choice for nonmalignant pain; however, dose reduction is mandated for frail older adults despite the pharmacokinetic and pharmacodynamicevidence for such a dose reduction being lacking. Animal studies have indicated a reduction in toxicity in old age, and this may possibly be the same for older frail adults. Understanding how ageing and frailty affect changes in drug clearance and toxicity will improve utilisation of this valuable analgesic and many other medicines by older adults.

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Research Article

The Effects of Old Age on Hepatic Stellate Cells

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Aging is associated with marked changes in the hepatic sinusoid, yet the effect of old age on hepatic stellate cells (HSC) has not been well described. Transmission electron microscopy and immunohistochemistry were used to study the effects of aging on HSC in livers from rats (3-4 mths versus 24–27 mths) and mice (2-3 mths versus 20–22 mths). Desmin-positive HSC doubled in old age in both mice and rats. Alpha-smooth muscle actin- (αSMA-) positive cells did not increase significantly and remained only a small percentage of desmin-positive cells. Electron microscopy revealed that old age is associated with HSC that have a substantial increase in the number of lipid droplets which are larger in diameter. There was also a marked increase of HSC that protruded into the sinusoidal lumen in old mice. In conclusion, old age is associated with hyperplasia of HSC that are not activated and are engorged with lipid droplets.

1. Introduction

Old age is associated with various changes in the hepatic sinusoid. These include thickening of the liver sinusoidal endothelial cell (LSEC) and loss of endothelial fenestrations, deposition of perisinusoidal basal lamina and collagen, and upregulation of proteins not usually seen in the young healthy liver such as von Willebrands factor and collagen [1, 2]. This has been termed age-related pseudocapillarisation. In addition, there are increased numbers of basally activated Kupffer cells (KC) that respond poorly to immune challenges [3]. As well as the LSEC and KC, the other main cell in the hepatic sinusoid is the hepatic stellate cells (HSC).

The HSC (or Ito cell) is a pericyte that resides in the extracellular space of Disse and has long cytoplasmic extensions that wrap around the LSECs. HSC contain many lipid droplets that are rich in vitamin A, which generates characteristic autofluorescence. In the early phases of many chronic liver diseases, HSC become activated and contribute to the fibrosis by producing extracellular matrix components such as collagen. In this activated state, HSC lose their lipid droplets and become myofibroblastic in appearance and stain positive for α-smooth muscle actin (αSMA) [4, 5]. It is unknown whether HSC contribute to the mild perisinusoidal fibrosis seen in old age. Furthermore, a recent study has shown that LSECs are involved in the regulation of activation of HSC [6] which raises the possibility that the age-related changes in the LSEC might impact on HSCs.

There have been only a few reports of the effects of old age on HSC. An early study found that HSC had more and larger lipid droplets in old rats [7], a finding that we also described more recently in nonhuman primates [8] and mice [9, 10]. Here, we investigated whether old age is associated with any change in the number or activation of HSC and quantified the changes in lipid droplets that accumulate in HSC in old age.

2. Methods

2.1. Animals. Rats were F344 aged 3–6 mths and 24–27 mths ($n = 5$ old, $n = 6$ young) obtained from the National Institute

Table 1: Transmission electron microscopic analyses of the effects of old age on HSC.

	Number of lipid droplets/HSC	Number of lipid droplets/HSC with nucleus	Diameter of lipid droplets in HSC (μm^2)	HSC protruding into sinusoidal lumen (number and % of total HSC)
Rats				
Young	6.76 ± 4.51	6.91 ± 4.59	1.08 ± 0.44	8 (7%)
Old	9.17 ± 6.35*	10.09 ± 6.38*	1.20 ± 0.50*	10 (10%)
Mice				
Young	2.41 ± 1.24	2.41 ± 1.24	1.83 ± 0.87	3 (5%)
Old	6.33 ± 3.55*	6.77 ± 4.31*	3.15 ± 2.19*	29 (51%)*

*$P < .05$.

of Aging (Baltimore, USA). Mice were B10 aged 2–3 mths and 20–22 mths ($n = 3$ in each group) that were maintained at the Centenary Institute (Sydney, Australia) in specific pathogen-free conditions and fed standard laboratory chow. The study was approved by the Sydney Southwest Area Health Service Animal Welfare Committee. After ketamine/xylazine anesthesia, liver tissue was harvested and fixed with paraformaldehyde for immunohistochemistry or with glutaraldehyde for transmission electron microscopy.

2.2. Transmission Electron Microscopy. Two blocks of glutaraldehyde-fixed liver tissue from each animal were cut and studied using a Philips CM10 transmission electron microscope. Approximately 20 HSC for each animal were photographed at 2,600 magnification and subsequently analyzed using ImageJ (http://rsbweb.nih.gov/ij/). The larger diameter of each lipid droplet contained in the cell cytoplasm and the area of each cell was determined. The number of lipid droplets per HSC was also analysed only in those sections where the HSC contained a nucleus in an attempt to compensate for the possibility that the sections were taken in different planes. In total we analyzed 680 droplets and 108 HSC from young rats, 806 droplets and 98 HSC from old rats, 170 droplets and 59 HSC from young mice, and 351 droplets and 57 HSC from old mice.

2.3. Immunohistochemistry. Staining was performed for desmin, which is a marker of HSC, and αSMA, which is a marker of activated HSC [11]. Liver specimens embedded in paraffin blocks were used. Sections from each block were stained for desmin (NCL-DER11 Novocastra 1 : 200) and αSMA (NCL-SMA Novocastra 1 : 200). Rat liver sections were processed using an automatic IHC Leica staining protocol on Leica Bond Polymer, Refine Detection (DS9800) with Bond DAB enhancer 30 mL (AR9432). EDTA buffer pH 9 was used for the antigen retrieval step for 30 min for the detection of desmin. Mice sections were stained manually. For desmin visualization, slides were incubated for 10 min in microwave with the same antigen retrieval buffer (EDTA pH 9). Sections were pretreated with 0.3% hydrogen peroxide for 20 min in PBS and avidin-biotin blocking solutions and then incubated with primary antibodies over night at 4°C. Antimouse biotinylated secondary antibody (1 : 150 Sigma, St. Louis, MO) was applied for 40 min at room temperature followed by peroxidase-conjugated streptavidin (40 min, 1 : 50 Sigma, St. Louis, MO). Peroxidase activity was visualized using 3,3′-diaminibenzidine. Three representative images for each section were taken at 200 magnification and analyzed using the software ImageJ. For each photograph, parenchymal area was calculated (large vessels were excluded) and desmin- and αSMA-positive nucleated cells were manually counted, and results were adjusted for 0.5 mm^2 area.

2.4. Statistics. The results are expressed as average ± standard deviation and comparisons performed using parametric and nonparametric, two-tailed Students t-test, depending upon the Schapiro Wilk test for normality (SigmaPlot v11).

3. Results

Table 1 shows the results of the transmission electron microscopic analysis and Figures 1 and 2 show representative pictures of stellate cells in young and old rats and mice. There were changes in HSC with old age in both rats and mice, although the changes were more dramatic in the mice. With old age, the number of lipid droplets per HSC increased, more so in mice. In addition, the average diameter of the lipid droplets increased in both the rats and mice, again more marked in the mice.

The frequency distribution of the diameter of the lipid droplets is shown in Figure 3. There appeared to be a normal distribution of the diameter of the lipid droplets in young animals, and the droplets were larger in mice than in rats. In old age, the distribution was skewed to the right with an appreciable number of much larger droplets.

Transmission electron microscopy also revealed that there was an increase in the percentage and number of enlarged HSC that resulted in protrusion of the endothelial lining into the sinusoidal lumen in old mice, but not in old rats.

Table 2 shows the results of the immunohistochemistry analysis and Figures 4 and 5 show representative pictures of desmin and αSMA staining in livers from young and old rats and mice. HSC were more numerous in mice than rats. With

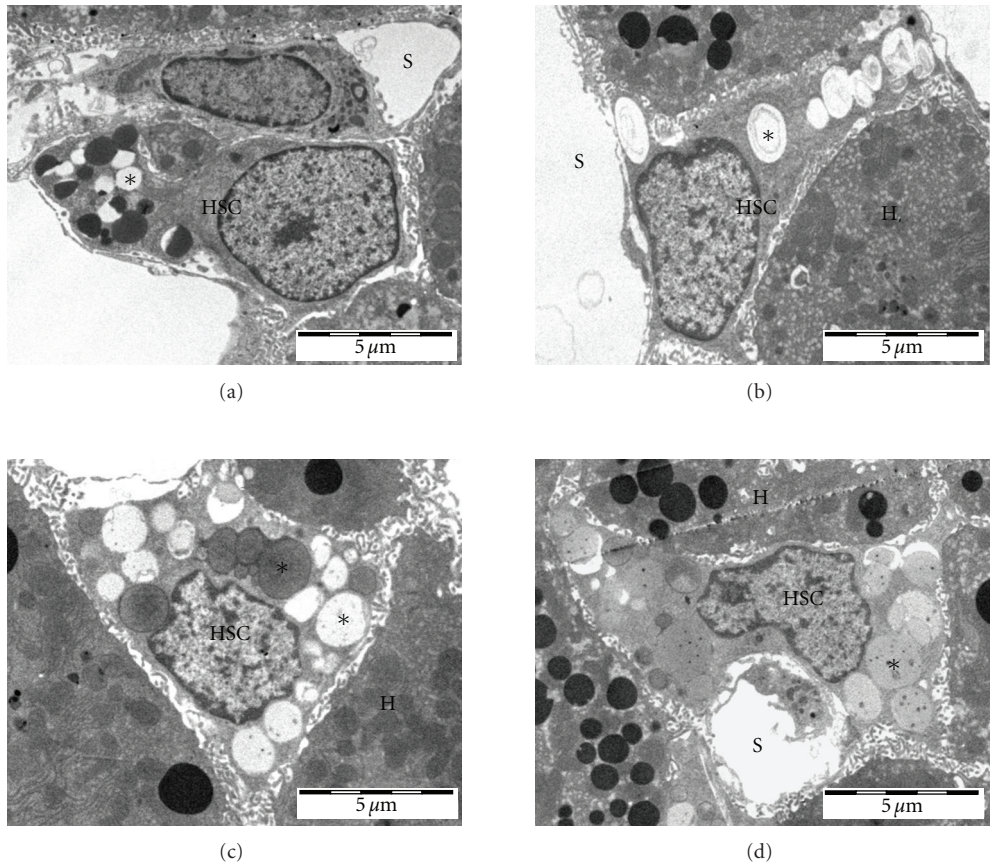


Figure 1: Representative transmission electron micrographs of HSC (HSC) from young (a,b) and old rats (c,d). Hepatic stellate cell, HSC; sinusoidal lumen, S; hepatocyte, H; lipid droplets*.

old age, there was an approximate doubling of the numbers of HSC. Activated HSC as determined by αSMA represented only a very small percentage of total HSC. Although there was an increase in αSMA-positive cells with old age in both rats and mice, this was not statistically significant nor did it represent a substantive change in the fraction of HSC that is activated. There was a small zonal variation in αSMA staining which was more intense in zone 2 (mid zone), but age did not have any impact on this distribution.

4. Discussion

Old age is associated with an increase in the number of HSC and and in the size and number of the lipid droplets that they contain, but no change in their activation status. Table 3 shows the results of all other studies that we could only find that make any mention of the effect of old age on HSC. There have been five other studies involving rats [7, 12], baboons [8], and mice [9, 10] that described changes in HSC lipid droplets in old age. In our study, we quantified these changes. In old age, there was an increase in the number of droplets per HSC by about 30% in rats and 250% in mice. The diameter of the droplets increased by about 10% in rats and 35% in mice. Lipid droplets in HSC contain about 80% of all retinoids in the body (vitamin A and its metabolites) mostly in the form of retinyl palmitate [4]. In addition, HSC lipid droplets contain triglyceride, cholesteryl ester, cholesterol, phospholipids, and free fatty acids. Retinyl ester and triglyceride are present at similar concentrations and together account for three-quarters of the total lipid in HSC lipid droplets [13]. In humans, plasma retinoids do not decrease with old age [14, 15], however, the postprandial levels are abnormal and consistent with reduced mobilization of vitamin A from the liver [15]. Vollmar et al. [16]

Table 2: Results of immunohistochemical study of the effects of old age on HSC.

	Desmin staining (number of positive HSC/0.5 mm^2)	αSMA staining (number of positive HSC/0.5 mm^2)
Rats		
Young	21.3 ± 15.1	0.6 ± 1.0
Old	45.5 ± 23.5*	2.8 ± 3.8
Mice		
Young	66.1 ± 28.8	0 ± 0
Old	134.0 ± 55.6*	1.1 ± 1.8

* $P < .05$.

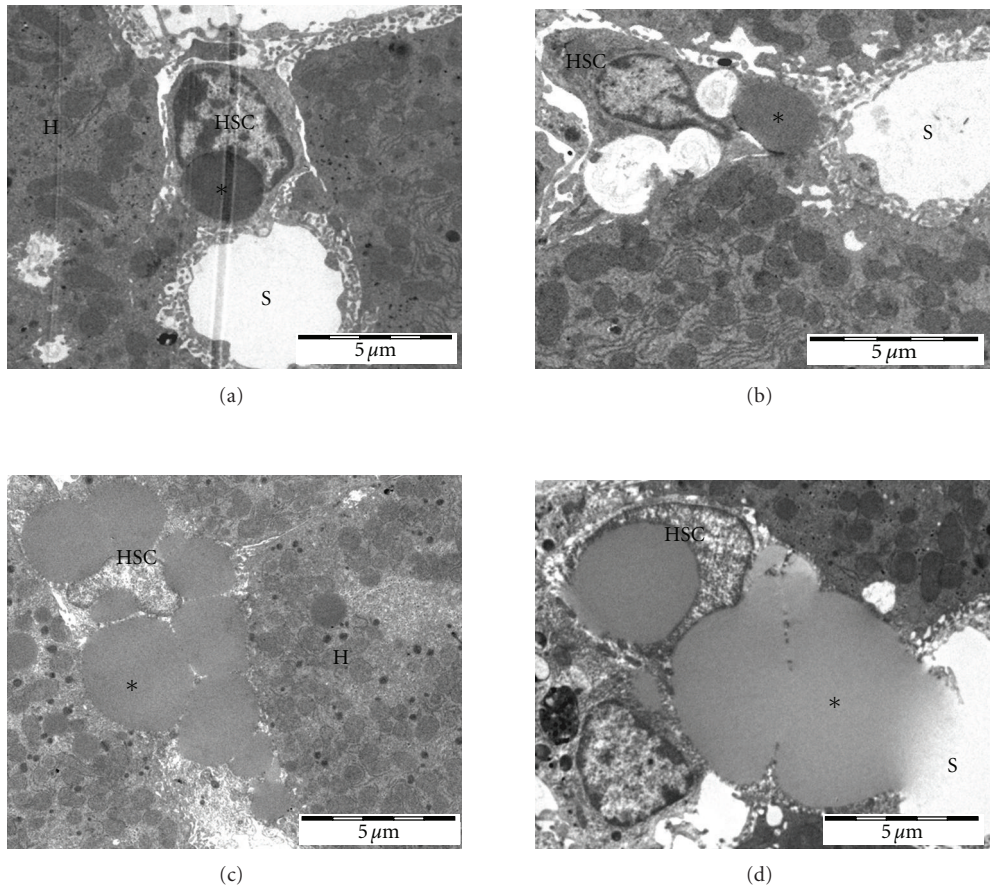


Figure 2: Representative transmission electron micrographs of HSC (HSC) from young (a,b) and old mice (c,d). Hepatic Stellate Cell HSC; Sinusoidal lumen S; Hepatocyte H; Lipid droplets*.

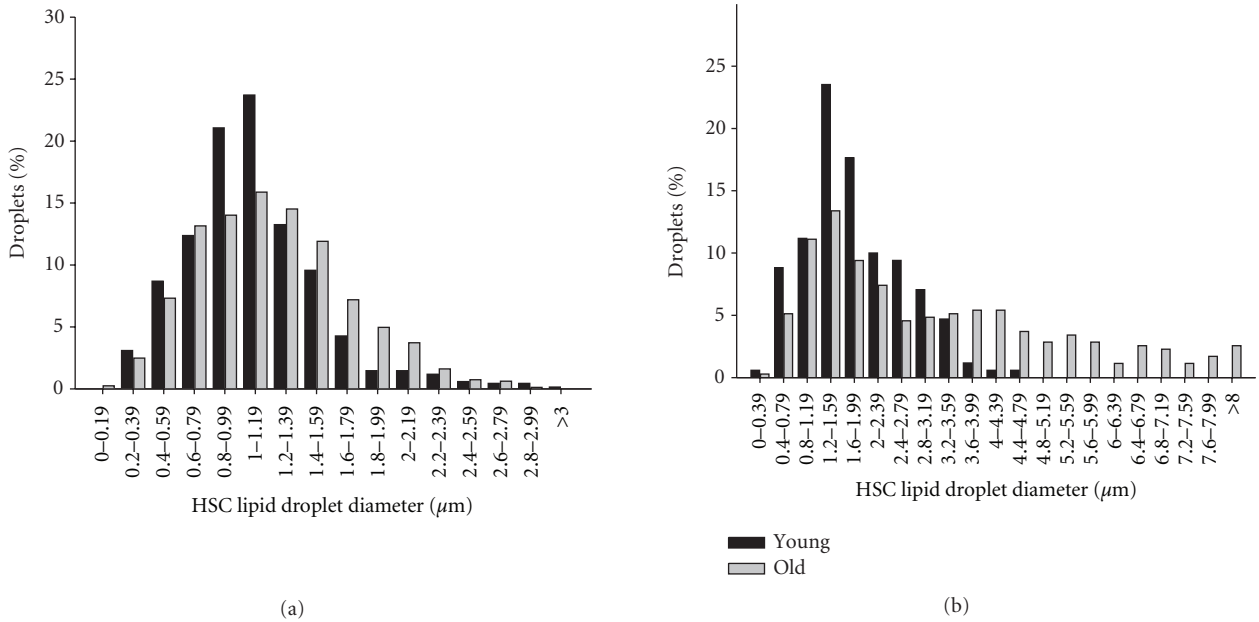
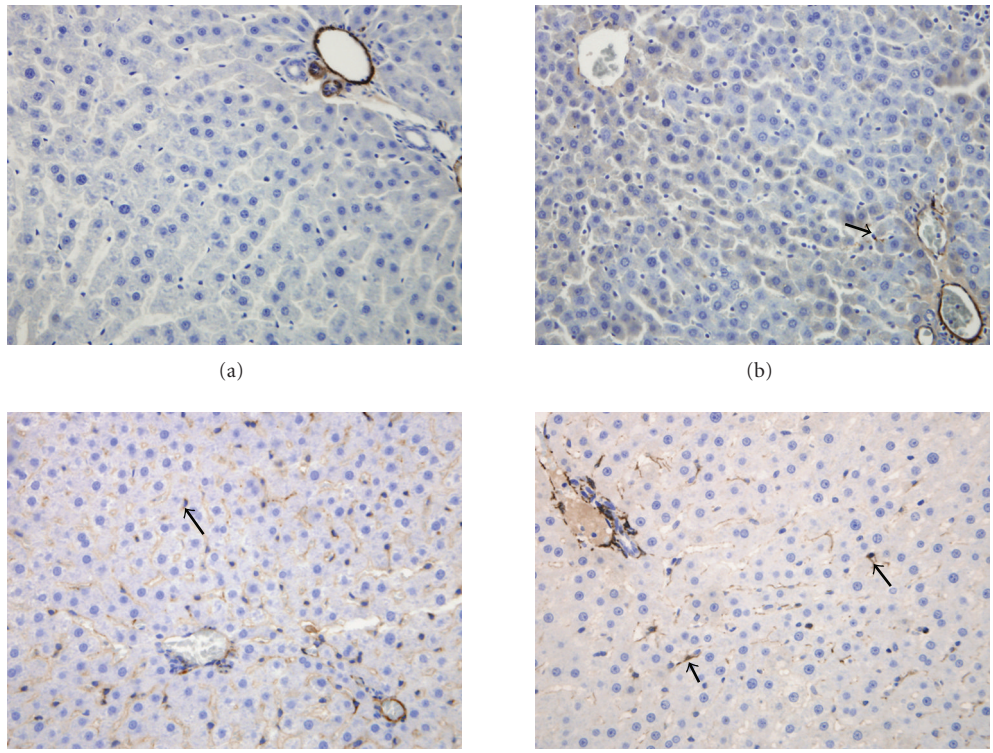


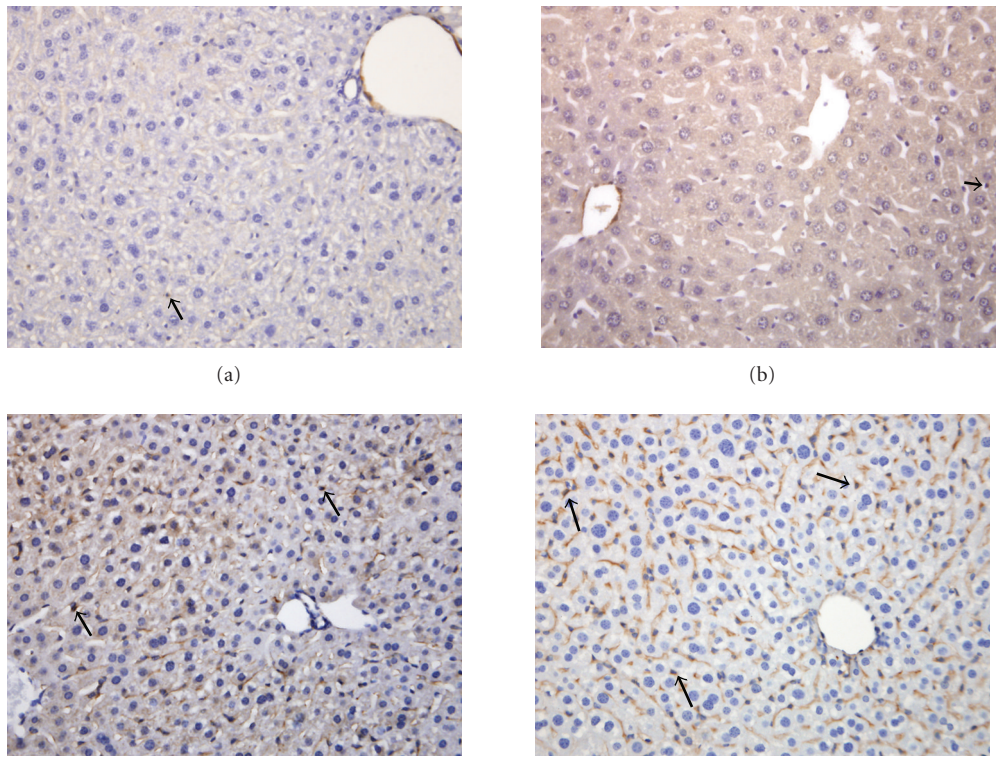
Figure 3: Frequency distribution of the diameter of the lipid droplets in HSC from rats (a) and mice (b). In both rats and mice, the distribution was skewed to the right in old age.



(a) (b)

(c) (d)

Figure 4: Representative immunohistochemistry from rat livers showing SMA in young (a) and old (b) rats, and desmin immunostaining in young (c) and old (d) rats (magnification ×200).



(a) (b)

(c) (d)

Figure 5: Representative immunohistochemistry from mice livers showing SMA in young (a) and old (b) mice, and desmin immunostaining in young (c) and old (d) mice (magnification ×200).

Table 3: The results of previous studies where the effect of old age on HSC was reported.

Citation	Species	Morphology	Number	Activation
[7]	Rat	↑ lipid droplets	↔	n.d.
[12]	Rat	↑ lipid droplets	n.d	n.d.
[18]	Rat	n.d.	↑ (from 0.43 to 1.02% of liver volume, $P = .075$)	n.d.
[16]	Rat	n.d.	↑ autofluorescence	n.d.
[8]	Baboon	↑ lipid droplets	n.d.	↔ (αSMA)
[19]	Rat	n.d.	n.d.	↔ (αSMA)
[9]	Mouse	↑ lipid droplets	n.d.	n.d.
[10]	Mouse	↑ lipid droplets	n.d.	n.d.

(n.d. not done).

reported an increase in retinoid content in old rat livers—a twofold increase of retinol, 32-fold increase of retinyl stearate, 53-fold increase of retinyl palmitate, and 66-fold increase of retinyl oleate. This suggests that the increase in the size and number of lipid droplets in HSC is related at least in part to an increase in retinoids in HSC. However, droplets also contain a similar amount of triglycerides, and there are reports of increased triglyceride content of old livers [17], but whether there is accumulation of triglycerides in HSC is not known.

There were increased HSC numbers in old age in both rats and mice. Previously, Enzan et al. [7] commented that there was no change in the frequency of HSC, but this study was performed using electron microscopy and no data were provided [7]. Similarly Martin et al. [18] concluded that there were no changes in HSC numbers in old age in rats. However, in their study, the volume of liver consisting of HSC increased from $0.43 \pm 0.13\%$ in young rats to $1.0 \pm 0.24\%$ in old rats but this did not reach statistical significance ($P = .075$ with only 4 rats in each age group) [18]. In our study, desmin immunohistochemistry was used to determine the number of HSC present in liver. In both rats and, mice old age was associated with approximately a doubling in the numbers of desmin-positive HSC per $0.5\,\text{mm}^2$. As such, the result is numerically consistent with that of Martin et al., [18] although our results were statistically significant. It is of interest that we have previously found an increase in the number of Kupffer cells in old age. Specifically, the number increased from 2.0 ± 0.2 to 5.5 ± 0.6 cells per $29{,}500\,\mu\text{m}^2$ in old age in rats [3]. The effect of old age on the numbers of the other main cell of the hepatic sinusoid, the LSEC is unknown.

Finally, we performed immunohistochemistry with αSMA to determine whether these HSCs are activated. Activated HSCs are characterised by loss of lipid droplets and expression of αSMA. The fact that we and others have noted that HSC from old animals are engorged with lipid droplets is consistent with the conclusion that HSC are not activated in old age. Likewise, we did not find any statistically significant change in αSMA-positive HSCs in old age. Although there was a slight increase in the number of αSMA-positive cells in old age, this was balanced out by the doubling in the total number of HSC so that the fraction of total HSCs that is activated remained only very small. Previously Grizzi et al. [19] found a nonsignificant increase in αSMA in the livers of old rats, but this did not increase in response to carbon tetrachloride as was seen with young livers [19]. We also noted that αSMA staining was not increased in the livers of old baboons [8]. The lack of activation of HSCs in old age means that it is unlikely that these cells are contributing to the mild perisinusoidal fibrosis that is seen in many species in old age [2]. Furthermore, the fact that HSCs are quiescent in old age suggests that the other changes of pseudocapillarisation seen in the aged hepatic sinusoid are unlikely to be secondary to undiagnosed chronic liver disease because this is invariably associated with activation of HSCs.

In conclusion, old age is associated with increased numbers of fat engorged, quiescent HSCs. This has implications for the effect of age on retinoid metabolism and for the mechanisms for other age-related morphological changes in the hepatic sinusoid. The increase in the percentage and number of enlarged HSCs that resulted in protrusion of the endothelial lining into the sinusoidal lumen in old mice may contribute to the reduced sinusoidal blood flow reported in aged mice [10].

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Review Article

Metabolism, Genomics, and DNA Repair in the Mouse Aging Liver

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The liver plays a pivotal role in the metabolism of nutrients, drugs, hormones, and metabolic waste products, thereby maintaining body homeostasis. The liver undergoes substantial changes in structure and function within old age. Such changes are associated with significant impairment of many hepatic metabolic and detoxification activities, with implications for systemic aging and age-related disease. It has become clear, using rodent models as biological tools, that genetic instability in the form of gross DNA rearrangements or point mutations accumulate in the liver with age. DNA lesions, such as oxidized bases or persistent breaks, increase with age and correlate well with the presence of senescent hepatocytes. The level of DNA damage and/or mutation can be affected by changes in carcinogen activation, decreased ability to repair DNA, or a combination of these factors. This paper covers some of the DNA repair pathways affecting liver homeostasis with age using rodents as model systems.

1. Introduction

The liver plays a pivotal role in the metabolism of nutrients, drugs, hormones, and metabolic waste products, thereby maintaining body homeostasis. The liver is central to glucose and lipid homeostasis as well as steroid biosynthesis and degradation. This organ also has a major impact on health and homeostasis through its control of serum protein composition. The liver undergoes substantial changes in structure and function in old age. For example, serum and biliary cholesterol rise, liver regeneration declines, hepatic drug clearance decreases, and liver volume and blood flow decrease with advancing age [1]. Such changes are associated with significant impairment of many hepatic metabolic and detoxification activities with implications for systemic aging and age-related disease. In addition to the altered hepatocyte functions with age, major morphological changes also occur with the liver sinusoidal endothelial cells. These specialized endothelial cells are very thin and contain pores approximately 50–150 nm in diameter grouped together in clusters. This fenestration of the endothelial lining allows a wide range of substrates including larger molecules like triglyceride-rich spherical lipoproteins called chylomicrons to reach the underlying hepatocytes for processing [1]. The size and number of pores decrease with age in several mammalian species. Such changes are believed to impact directly on the hepatic metabolism of lipoproteins thus predisposing aged individuals to cardiovascular diseases [2]. Finally, all these morphological and functional changes in the liver tissue are likely to affect therapeutic interventions due to the handling and metabolism of pharmacological agents in older individuals. Adverse drug reactions are estimated to be two to three times higher in elderly patients than in young adults [3, 4].

Concomitant with morphological changes, the liver exhibits important alterations in global gene expression profiles with age. In mice, aging is accompanied by changes in expression of genes associated with increased inflammation, cellular stress, fibrosis, altered capacity for apoptosis, xenobiotic metabolism, normal cell-cycle control, and DNA replication [5]. Lifelong calorie restriction reversed the

majority of these changes [6]. In a recent study with C57BL/6J mice, the expression profiles of liver tissues from eight-month-old and 32-month-old animals were compared [7]. Aging was accompanied by suppression of genes involved in the insulin growth factor-1/growth hormone pathways, carbohydrate metabolism and ATP biosynthesis, xenobiotic metabolism, and peroxisomal biogenesis. Finally, old animals exhibited an increase in the immune and inflammatory responses and an upregulation of genes associated with protein and amino acid glycosylation. Overall, liver from old mice exhibits a decrease in growth/proliferation pathways and metabolisms that would increase oxidative stress, and an increase in inflammatory and stress response genes.

Several theories have been proposed for the age-associated alterations observed in the liver, and one of them is the somatic mutation theory of aging. This theory was followed by the DNA damage theory of aging [8], which proposes that the accumulation of genomic instability in cells of a tissue, particularly in the stem cell compartment of the tissue, will modify several genes that can eventually lead to either cancer, apoptosis, senescence, or abnormal tissue homeostasis. A body of correlative data supports this theory, but how much genetic instability contributes to overall aging is still in debate. Nonetheless, it is important to mention that even though DNA repair may not change with age, mutations may still accumulate with the number of cell division in a tissue due to unavoidable DNA replication errors. In a situation in which DNA repair declines with age, unrepaired DNA lesions may persist with harmful consequences to the cells with age. Unrepaired DNA lesions may not only affect the effectiveness of copying chromosomes during DNA replication, but also the transcription of damaged loci. Finally, a shift from error free to error-prone DNA repair pathways during aging may also contribute to the accumulation with age. In this paper, we will use the mouse model to describe what is known about DNA repair in the aging liver and its probable implication in the age-associated physiological modifications.

2. Mutation Rate in the Liver of Mice with Age

The mouse is a good biological tool that allows the analyses of different tissues with little limitation on the amount of biological materials available. Mice are economical compared to larger mammals, and there is a huge volume of literature on the physiology, behavior, and biochemistry of such rodents. Importantly, it is possible to modify the diet of mice or treat them with drugs to mimic specific diseases and/or to improve their health status. Finally, their genomics and genetics have been extensively studied to such a point that now there is a battery of transgenic and knockout mice which, to some extent, phenocopy important age-related diseases. Many mice with mutations in different DNA repair proteins are available. Importantly, at least four transgenic lines with the *lacI* and/or *LacZ* reporter genes have been intensively used to estimate the mutation frequency or rate in the genome of different tissues with age. One such transgenic line bears a lambda shuttle vector that carries a *lacI* target and an alpha *lacZ* reporter gene [9, 10]. Genomic DNA is isolated from the tissue under study, and the shuttle vector is recovered by exposing the DNA to lambda phage packaging extracts *in vitro*. Mutations in the *lacI* target gene that inactivate the repressor gene allow expression of the alpha *lacZ* reporter gene, resulting in blue mutant plaques. Sequencing of the DNA from these plaques not only allows the estimation of the mutation frequency, but it also points to the type of mutation providing insights into potential mechanisms [9, 10]. The *lacI* gene is highly sensitive to base substitution and frame shift mutations, as well as small deletions and insertions, making the transgene an ideal choice for recovery of spontaneous and induced mutations [11, 12]. The Big Blue mouse contains approximately 40 copies of the lambda shuttle vector stably integrated as a tandem array at a single position in chromosome 4 [12]. The MutaMouse contains the sequence of a phage carrying the *lacZ* gene integrated in a head-to-tail arrangement of approximately 40 copies located at a single insertion site in chromosome 3 [13, 14]. The technical difference in identifying mutations in these two mouse systems is that the Big Blue mouse model is based on forward mutations in the *lacI* reporter sequence derepressing the *lacZ* gene thereby yielding blue plaques as mutants. Thousands of plaques need to be examined. The Muta mouse is based on forward mutations in the *lacZ* reporter gene that can be easily selected because only mutants will generate plaques. Finally, the *lacZ* transgenic mice lines 30 and 60 bear a plasmid carrying the *lacZ* gene. Line 60 was found to have two integration sites, which were mapped to chromosomes 3 and 4. The plasmid integration site of line 30 is on chromosome 11. Each integration site in both transgenic lines has about ten to twenty plasmids per haploid genome [15]. Plasmids are rescued by excision with the restriction enzyme HindIII, followed by separation from mouse genomic DNA by the use of magnetic beads coated with the lacI repressor protein, which will bind the *lacI* sequence. The recuperated DNA is then self-ligated to obtain circular plasmids that are finally transferred into *Escherichia coli* C bacteria (harboring a deletion of its own *lacZ* gene) for sequence analyses [15, 16]. Mice of line 60 are appropriate transgenic animals for the study genome rearrangements in the aging liver [15, 17], and chromosomal translocations and deletions up to 66 megabases have been observed in the tissues of such mice [17]. Such chromosomal rearrangements cannot be detected using the phage-based reporter models (the MutaMouse and the Big Blue models).

Before describing the findings obtained with these transgenic mouse mutation detection systems, one needs to have an overview of liver growth and development. The following information was obtained from a paper published by Stuart and Glickman in 2000 [10]. The liver is regarded as a slowly renewing tissue. The liver from two-week-old CBA/C57BL mice contains approximately 0.8×10^8 hepatocytes/cm^3 of tissues. This number increases by 1.5-fold to approximately 1.2×10^8 cells in three-month animals, then decreases slightly to 0.98×10^8 and 0.88×10^8 cells/cm^3 at ages of 12 and 24, respectively. Cell proliferation decreases significantly (approximately 3.3-fold) in the liver of male mice from

ages 2.5 to 32 months. Although the number of cells in the postnatal liver reaches a plateau, DNA synthesis continues at a reduced rate throughout adulthood, resulting in an age-related increase in mean polyploidy. Thus, mean DNA ploidy levels in mouse liver double from ages of one week to one month and thereafter increase steadily, doubling again by 24 months of age. This increase in liver polyploidy is accompanied by an increase in liver weight but not in cell number. From the age of two to three weeks, it has been reported that each liver cell in the mouse enters the mitotic cycle from one to six times (three on average), resulting in an eight-to-ten-fold increase in liver mass and about a threefold increase in the number of cells. Mature hepatocytes are fully differentiated, self-maintaining cells with low proliferative rate and low, if any, rate of cell elimination from the population during the life of the mouse. The liver cells in newborn mice are diploid but polyploidy levels increase in young animals [10]. The presence of advanced polyploidy in mammalian cells is generally considered an indication of terminal differentiation and senescence of cells [18]. As pointed out by Gupta in 2000 [18], although the existence of a liver stem cell is often debated, most experts agree that progenitor liver cells are activated in response to significant depletion of hepatocytes following exposure to hepatotoxins or carcinogens. It has been estimated that mouse hepatocytes have a turnover time of 480–620 days [10]. Thus, an adult mouse would have up to 30–40% of hepatocytes with more than one nucleus. Multinucleated hepatocytes are thought to develop in response to completion of DNA synthesis and mitosis but with failure of cell division or cytokinesis, which would normally generate daughter cells containing single nuclei [18]. The exact molecular mechanisms leading to polyploidy in the liver are still unknown. Recent evidence, however, suggested that key components of the insulin pathway (PI3K and Akt kinases) control cytokinesis failure events. For example, rats injected with insulin exhibit increased tetraploidization in the liver [19]. Interestingly, polyploid hepatocytes are under oxidative stress, as suggested by an antioxidant depletion, increased lipid peroxidation, and elevated 8-hydroxyguanine in nuclear DNA. Accordingly, introduction of oxidative injury significantly induced polyploidy in cultured hepatocytes freshly isolated from rat liver [20]. Liver repopulation assays (after partial hepatectomy) in rodents also show markedly decreased replication capacity in polyploid hepatocytes [20]. Thus, the diploid cells will be responsible for liver regeneration after a partial hepatectomy as they still retain their full proliferative potential unlike polyploidy cells. The presence of senescence-type changes in polyploidy hepatocytes is likely to affect normal liver homeostasis during aging. In addition, cell polyploidy is a potential source of aneuploidy, DNA rearrangements, and tumorigenesis. Indeed, chromosomal aberrations after partial hepatectomy increase with aged mice. Additional results based on the analyses of the *lacZ* reporter plasmid (transgenic mouse line 60) showed a rapid accumulation of genome rearrangements in the liver of old mice (27 months of age and older) [15]. On average, there was a two-to-four-fold increase in liver genome rearrangements involving the *lacZ* reporter gene over the life span of mice [15, 17]. The authors of these studies hypothesized that the increase in DNA rearrangements in the liver of old mice was due to oxidative stress [15].

The free radical theory of aging, first proposed by Harman in 1956 [21], has received a lot of attention over the years as indicated by the number of scientific reviews on antioxidant interventions in different animal models and human clinical trials. The mitochondrion has been identified as a major source of reactive oxygen species (ROS) and thus oxidative stress potentially contributing to the aging process, although several plasma membrane and cystosolic enzymes may also contribute to the increased intracellular pro-oxidant status observed during aging [22]. In the mitochondrial respiratory chain, electrons entering complexes I and II are transferred to complex III, then IV where they are combined with molecular oxygen and hydrogen to form H_2O. Redox reactions at respiratory complexes I, III, and IV are coupled to the extrusion of protons from the mitochondrial matrix into the intermembrane space. The re-entry of protons into the matrix is coupled to the synthesis of ATP from ADP and P_i. This oxidative phosphorylation is responsible for the vast majority of ATP production and oxygen consumption in most types of animal cells [23]. Up to 2% of oxygen used in this complex reaction undergoes monoelectronic reduction and results in the formation of superoxide anion and hydrogen peroxide, which can lead to the formation of the more toxic species hydroxyl radicals [24, 25]. Such reactive species can attack and modify genomic DNA. An important type of oxidative DNA lesion accumulating with age is 8-oxo-deoxyguanine [26]. If unrepaired, this adduct in genomic DNA may lead to a point mutation upon DNA replication. During DNA replication, 8-oxo-deoxyguanines present on either strand of DNA can mispair with adenosines and lead to G:C → T:A transversion mutations. A misincorporation of an 8-oxo-deoxyguanine as a substrate nucleotide can also lead to the same type of mutational pattern [27].

The Big Blue transgenic mouse detection system was used to examine the spontaneous point mutation frequency and pattern in the aging liver [9, 10, 12, 28]. The major conclusions from these studies are that the spontaneous mutation frequency increased with age in mouse liver, although no specific type of point mutation predominated (transversion or transition type of mutation) with age. The only exception was an increase in the frequency of GG → TT tandem-base mutations, which specifically increased with age in the liver. Although there was a lot of interindividual variations between old mice, overall the liver showed approximately two-to-four-fold increase in point mutation frequency from 10 to 30 months of age [28]. In contrast, the GG → TT tandem transversion mutation in the liver of Big Blue mice increased eight-fold in middle-aged animals (compared to young mice) and 20-fold by 25 months of age [12]. The GG → TT tandem-base mutation represented a mutational signature of an age-related change in older liver not seen in other tissues. The increased frequency of endogenous GG → TT tandem-base mutation pattern in the aging liver may be due to mutagenic acetaldehyde derivatives, which

can result from lipid peroxidation reactions [12], as the liver is the major site for lipid metabolism in the body [2]. Notably, the mutation frequency and pattern (type of mutations) in other tissues, like brain or germline tissues for example, were different than in liver [9, 10, 12, 28]. This conclusion was also reached with *lacZ* transgenic mouse model [29].

In view of the low mutation frequency in the liver and the low levels of DNA rearrangements, several authors argued that such changes in the genetic material would not be sufficiently high to have an impact on liver function with age. It is possible, however, that the transgenic mouse mutation detection system is underestimating the number of persistent DNA lesions (or damage) that would affect DNA replication or transcription. They also miss mutations that only decrease gene activity, but which can still have an impact on aging. Finally, the transgenic reporter systems cannot detect mitotic recombination events that can lead to somatic mutations [30].

3. Levels of DNA Damage in the Mouse Aging Liver

As discussed above nucleic acids can be oxidized by ROS that are formed as natural byproducts of the mitochondrial metabolism of oxygen. In addition, organisms are constantly exposed to exogenous sources of ROS and environmental agents or pollutants that can damage DNA. The most common pollutants include car exhaust particles, cigarette smoke, UV and ionizing radiation, insecticides, and pesticides, among others. The most characterized mutagenic DNA lesion appearing due to endogenous metabolism is 8-oxo-deoxyguanine. The presence of this lesion in the DNA results from either the direct oxidation of guanine bases in the DNA or as a consequence of incorporation of 8-oxo-7, 8-dihydro-2′-deoxyguanosine 5′-triphosphate (8-oxodGTP) during replication [31]. Several publications have indicated that mice accumulate 8-oxo-deoxyguanine lesions in their liver with age by approximately 1.8-to-2.8-fold [31–34]. Concomitant with the increase in 8-oxo-deoxyguanine lesions, persistant single-strand breaks have also been described in the liver of aging mice [32]. This is, however, still debated as there are several reports indicating no increase in 8-oxo-deoxyguanine lesions or single-strand breaks in the liver of old rodents compared to young animals [35, 36]. It has been suggested that the discrepancies observed in the literature are due to artifactual DNA oxidation, which arise during the isolation and analysis of the DNA samples [34], and a consortium of laboratories (the European Standards Committee on Oxidative DNA Damage, ESCODD) was established to look into these methodological aspects [37].

Formamidopyrimidines such as 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 4,6-diamino-5-formamidopyrimidine are also DNA lesions occurring due to oxidative stress related to mitochondrial metabolism [31], which can be detected in liver DNA at levels comparable to or even higher than those of 8-oxo-deoxyguanine in six-month-old animals [38]. In contrast to 8-oxo-deoxyguanine lesions, however, formamidopyrimidine lesions did not increase in the liver of old mice [31]. It has been estimated that 8-oxo-deoxyguanine makes up approximately 5% of all oxidative lesions [39]. Although the steady-state levels of 8-oxo-deoxyguanine would seem relatively small in the genome of rodent cells, the accumulation of 8-oxo-deoxyguanine in specific sites in the genome is expected to be highly relevant for aging.

Telomeres are DNA structures composed of TTAGGG repeats of several kilobases (thus G rich sequence) required for the maintenance of chromosomal ends. They protect chromosomes from end-to-end fusion, recombination, and degradation. It is now well accepted that an increase in chromosome instability may be associated with loss of telomeric repeats and cellular senescence [40, 41]. Senescent cells are often oblivious to external growth stimuli and may affect the rejuvenation or normal homeostasis of tissues with age. Thus, due to its high guanine content, the telomeres are hot spots for oxidative damage, at least in human cells [42]. More importantly, 50% of single-strand breaks remain unrepaired in telomeres unlike the same kind of damage in other part of the genome [42]. Embryonic fibroblasts from mice lacking the DNA glycosylase OGG1, which removes 8-oxo-deoxyguanine, showed telomere shortening and increased sister chromatid exchange, indicating an important role of this lesion in telomere dysfunction [43]. Finally, it has been reported that a single 8-oxo-deoxyguanine lesion in a defined telomeric substrate reduced the percentage of bound TRF1 and TRF2 proteins by at least 50%, compared with undamaged telomeric DNA [44]. Thus, oxidative DNA damage may also exert deleterious effects on telomeres by disrupting the association of telomere maintenance proteins TRF1 and TRF2 [44]. "Uncapped" telomeric 3′-overhang sequence TTAGGG will induce a senescent phenotype in cultured human fibroblasts [45]. Despite the observation of telomere attrition in OGG1-deficient mice, such mice do not exhibit a premature aging syndrome. These results suggest that other unknown processes are at play during aging or that the extent of telomere dysfunction is not as high in tissues as one would see after several generation in mice lacking the enzyme telomerase required for telomere maintenance [46].

A recent study has indicated a good correlation between the presence of senescent cells in the liver of mice (detected on cryosections with the senescence associated β-galactosidase) and the presence of DNA breaks detected by immunohistochemistry with an antibody against the double-strand DNA break marker γ-H2AX [47]. The vast majority of γ-H2AX foci-positive cells in the liver were hepatocytes as judged by morphological criteria. The frequency of γ-H2AX foci-positive cells increased significantly with age by twofold in the liver of mice. More precisely, γ-H2AX foci-positive cells increased from 17% of the hepatocytes in the liver of 12-month-old mice to 34% of hepatocytes in 42-month-old animals [47]. At all ages, however, the frequencies of foci-positive hepatocytes in the periportal areas were lower than in the centrilobular and intermediate areas of the lobes. Thus, some regions of the mouse liver are more likely to accumulate

DNA breaks with age. It is worth mentioning that not all γ-H2AX foci-positive cells in the liver of mice are also positive for senescence-associated β-galactosidase [47]. The potential problems with γ-H2AX foci-positive cells are that they can die by apoptosis or accumulate carcinogenic lesions. Dead cells must be replaced by a constant renewing of stem cells. If stem cells are senescent and do not respond to environmental stimuli to divide, the accumulation of senescent cells with age will eventually affect liver rejuvenation and the normal homeostasis of this tissue.

There is evidence for an increase susceptibility to DNA damage in the liver from exogenous toxic agents (including oxidative stressors) with age. For example, the number of 8-oxo-deoxyguanine lesions in hepatic DNA of 14-month-old mice treated with carbon tetrachloride, known to generate ROS in the mouse liver, was significantly higher than that of the two-month-old animals treated with the same chemical [33]. Similarly, DNA lesions (mainly single-strand breaks) induced by the oxidizing agent nitroquinoline-N-oxide increased with age in the liver of mice [32]. Hepatic DNA from older mice are also more prone to alkylating damage than younger animals. In one report, liver DNA in old (29 months) mice accumulated 50% more damage when treated with the alkylating agent N-methyl-N-nitrosourea (MNU) than young (nine months) animals treated with the same amount of the drug (doses normalized on weight) [48]. N-nitroso compounds are widely disseminated in the environment and are important food contaminants. A major mutagenic DNA lesion produced by MNU in the liver of mice is the 7-methylguanine. Interestingly, the same study with MNU also revealed age-related changes in chromatin composition or structure that make some genomic sequences more accessible to alkylating agents in liver tissue of older animals [48]. Liver chromatin can be fractionated into nuclease-soluble, low-salt, high-salt, and nuclear matrix fractions. All fractions of liver chromatin from young mice (nine to eleven months) were equally modified by MNU. In contrast, nuclease-sensitive regions of liver chromosomes from old mice (28-29 months) were preferentially alkylated by MNU over bulk chromatin and nuclear matrices [48]. With regards to liver chromatin, it is worth mentioning that there is an age-dependent increase in the amount of DNA protein cross-links in some strains of short-lived mice compared to long-lived mouse strains [49]. The reason for this difference has yet to be determined.

Finally, the metabolism of procarcinogens in the liver will impact on the levels of DNA damage with age. For example, the genotoxicity of the environmental pollutant polycyclic aromatic hydrocarbon benzo[α]pyrene is dependent on its metabolic activation by liver P450 cytochrome monooxygenase enzymes [50]. The reactive metabolite binds predominantly to guanines in the DNA. Such DNA adducts are mainly repaired by the nucleotide excision repair pathway. Interestingly, the formation of DNA adducts by benzo[α]pyrene was decreased by approximately threefold in the liver of 18-month-old animals compared to two-month-old mice [50]. This decrease is concomitant with the reduced total microsomal P450 content observed in rodent liver tissue with age [51].

4. DNA Repair Enzymes in the Liver of Old Mice

DNA repair is required for genome stability. If lesions in DNA were not repaired, they would accumulate to high levels, incompatible with normal conditions of life. Cells have evolved a complex network of repair systems for the removal of DNA damage. If unrepaired, DNA lesions can lead to DNA polymerase arrest, which can result in double-strand breaks upon DNA replication collapse or breaks at regions of the genome that are actively transcribed and mutations. Both strand breaks and mutations accumulate with age in several model organisms, indicating an important role for DNA repair in preventing age-associated genomic instability. Because the chemical nature of the DNA lesions is varied, each type or class of DNA modification is repaired by a specific repair pathway. Small base modification, such as 8-oxo-deoxyguanine, many alkylated bases, and single-strand breaks are mainly recognized by enzymes of the base excision repair pathway (BER). Bulky covalent lesions, such as those induced by UV and several carcinogens, are repaired by the nucleotide excision repair pathway (NER). Double-strand breaks and interstrand DNA cross-links, on the other hand, require a specialized repair pathway and are repaired by non-homologous endjoining or by homologous recombination. It is noteworthy, however, that while these repair pathways have been characterized independently, *in vivo* there is substantial crosstalk among them. Some proteins involved NER may, in some cases, also participate in the repair for certain oxidative lesions, mostly via protein interactions with canonical BER proteins.

4.1. Base Excision Repair in the Liver of Mice. Base excision repair (BER) functions by glycosylase-initiated removal of a damaged base followed by incision of the DNA backbone, synthesis of new DNA, cleaning up of the 3′ and 5′ ends, and ligation [52]. Various DNA glycosylases recognizing specific types of damage initiate BER. Incision of the phosphate backbone is accomplished by an AP endonuclease, APE (HAP1, APEX, REF1). In the nucleus, the DNA synthesis step in the BER pathway is carried out by DNA polymerase β (β-pol) [53]. It is estimated that 70–90% of all BER takes place via the replacement of a single nucleotide by β-pol, short-patch pathway [54]. In a minor subpathway termed long-patch BER, β-pol is believed to initiate repair synthesis and can replace up to six nucleotides [55]. The long-patch BER is also DNA polymerase δ/PCNA- and FEN1-dependent [56, 57]. Poly(ADP-ribose) polymerase 1 (PARP1), functioning as DNA nick-sensor, will regulate the activity of β-pol during long-patch BER [58]. In 2002, Cabelof and colleagues [52] reported their analyses of uracil-initiated short-patch BER in liver extracts of C57BL/6 mice at different ages. The G:U mismatch is estimated to be responsible for 70–90% of all BER [54]. Nuclear extracts from the liver of 22–26 month-old mice showed a 50–75% decrease in the repair of a DNA duplex containing G:U mismatch compared to the liver of young four-to six-months-old animals [52]. A similar decrease in the liver of old mice was also obtained with a DNA duplex containing an 8-oxo-deoxyguanine, although no changes in OGG1 activity in the nucleus of mouse liver

with age had been observed previously [59]. Interestingly, they found a decrease in β-pol protein levels in the liver of old C57BL/6 mice compared to young mice. The group also examined the *lacI* mutation frequency in response to the alkylating agent dimethyl sulfate in their young and old animals. The mutation frequency did not significantly increase in young animals whereas identical exposure in aged animals resulted in a fivefold increase in mutation frequency. Because dimethyl sulfate induces DNA damage processed by the BER pathway, the authors suggested that the increased mutagenicity of dimethyl sulfate with age was related to a decline in BER capacity that occurred in the liver of aged mice [52]. The predominant lesion induced by dimethyl sulfate is N^7-methylguanine, which is efficiently repaired by BER. Because the glycosylase activity for N^7-methylguanine does not appear to decrease during aging [60], it is likely that the decrease in BER observed in old C57BL/6 mice is due to a decrease in β-pol activity. The following year, Intano and colleagues [61] also observed, in two different mouse strains (outbred CD1 and hybrid $B_6D_2F_1$ mice), a 50% decrease in G:U mismatch repair in nuclear extract from the liver of aged mice. They were unable, however, to see a difference in β-pol protein levels in the liver of young and old mice (3-, 18-, and 28-month-old mice). The levels of other proteins important for BER of G:U mismatch such as ligase I and III, XRCC1, APE/REF-1 were not changed with age [61]. The difference in β-pol protein levels in C57BL/6 mice with age could be mouse strain-specific, or it could even be due to differences in husbandry conditions [62].

Removal of 8-oxo-deoxyguanine in chromosomes is primarely initiated by the 8-oxoguanine DNA glycosylase 1 (OGG1) or, to a lesser extent, Nei endonuclease VIII-like 1 (NEIL1) repair enzymes [31]. In the nucleus, the expression of OGG1 and NEIL1, at least at the mRNA level, does not change with age in the liver of C57BL/6 mice. Similarly, 8-oxo-deoxyguanine glycosylase activity was not significantly changed with age in mouse or rat liver [59, 63]. In the nucleotide pool, 8-oxodGTP is generated by the oxidation of 2′-deoxyguanosine 5′-triphosphate (dGTP). One of the nudix enzymes (nucleoside diphosphate-linked moiety X)-type motif 1 (Nudt1, also called MTH1) degrades 8-oxod GTP to 8-oxodGMP, thereby preventing its incorporation into chromosomes during DNA replication [31]. The mRNA levels of Nudt1 do not change with age in mouse liver. Intriguingly, the 8-oxodGTPase activity in the liver significantly decreases by approximately 30% from six to twelve months compared to two-to-three-month-old mice. It then increases back to levels observed in the liver of two-three-month-old animals when mice reach the age of 25 months [31], although the levels of 8-oxo-deoxyguanine lesions were still higher in the liver of 25-month-old animals [31]. The authors suggested that the increase in 8-oxodGTPase activity observed in older animals could be an adaptive response to an increase in endogenous ROS production that can potentially overwhelm the antioxidant systems of aged hepatocytes. Food consumption per body weight is also higher in two-to-three-month-old mice compared to six-to-twelve-month olds, potentially contributing to higher endogenous metabolic ROS in young animals. Appropriate experiments are warranted to confirm this hypothesis. Overall, oxidative DNA damage increases in the liver of mice with age concomitantly with a decline in BER. Knockout of several BER enzymes has been generated in mice over the years. A comprehensive review on this subject has been already published by Xu and colleagues and will not be discussed here [62].

4.2. Nucleotide Excision Repair in the Liver of Mice. The nucleotide excision repair (NER) is the main DNA repair system for the removal of bulky and helix distorting lesions in DNA. The NER pathway is composed of a multiprotein complex that removes an oligonucleotide containing the lesion. The 5′-incision, which is carried out by an endonuclease complex containing the excision repair cross-complementing rodent repair deficiency, complementation group 1 (ERCC1) protein, is the first step in the excision of the nucleotide and can be considered as the rate-limiting step of the NER process [31]. The mRNA levels of Ercc1 do not change with age in the liver of mice [31]. One study, however, has indicated a significant decrease in NER activities in hepatocytes from 24-month-old rats compared to hepatocytes from six-month-old rats [64]. This difference with age, though, was specific to certain regions of the genome. Accordingly, there are age-related changes in the structure and function of the chromatin in different regions of the genome, which may affect the efficiency of DNA repair [65, 66].

The expression of several proteins involved in the NER pathway was found to decrease with age in the skin of mice [67], although the same has not been observed in the mouse liver tissue. Nonetheless, knockout of several NER proteins has been generated in mice over the years [68], recapitulating some of the human segmental progeroid syndromes like Xeroderma pigmentosum, Cockayne syndrome, or trichothiodystrophy [68]. Several of these mice exhibit, in addition to an accumulation of mutations in their liver tissues, transcriptional changes related to metabolic abnormalities or phenotypes associated with aging (discussed below).

4.3. Repair of Double-Strand Breaks in the Liver of Mice. Two major repair pathways are involved in the repair of double-strand breaks in order to preserve genomic integrity: the non-homologous end joining (NHEJ) and homologous recombination (HR). The NHEJ system repairs double-strand breaks in the DNA by joining free ends together without the aid of a homologous template and occurs during the G_1 and S phases of the cell cycle. This reaction is therefore prone to errors during repair. The major proteins involved in this pathway include Ku70, Ku80, DNA-PK_{CS}, Artemis, XRCC4, DNA Ligase IV (LIG4), WRN, and the XRCC4-like factor [69]. Ku70 and Ku80 form a heterodimer that binds to DNA ends and together with a PI-3 kinase catalytic subunit, DNA-PK_{CS}, forms a holoenzyme referred to as DNA-PK (DNA-dependent-protein kinase). Artemis opens hairpins and processes overhangs in a complex with DNA-PK_{CS}, thereby generating substrates that are ligated by the XRCC-LIG4 heterodimer in a complex with the XRCC4-like factor [69]. Finally, the exonuclease/DNA helicase WRN

protein cooperates with the XRCC4-DNA ligase IV complex during end-processing [70]. Importantly, many proteins of the NHEJ reaction are required for the maintenance of the telomeric ends of chromosomes, including Ku70, Ku80, DNA-PK_{CS}, and WRN [71]. These proteins will impact telomere length maintenance and will suppress telomere fusions that could eventually lead to chromosome rearrangements [69, 71].

In the HR reaction, the sister chromatid or homologous chromosome is used as a template to repair the broken DNA. Thus, HR is largely restricted to late G2 and M phases of the cell cycle, when these are available after DNA replication [71]. The first step in HR is processing of the double-strand break by a nuclease to generate 3′ single-stranded DNA tails, which are coated with the single-strand binding protein RPA. The MRN complex, composed of the proteins MRE11, RAD50, and NBS1, is a candidate for this nuclease, although other nucleases are likely involved as well. The RAD51 protein, assisted by a number of factors including RAD52, RAD54, BRCA2, and the RAD51 paralogs (XRCC2, XRCC3, RAD51B, RAD51C, and RAD51D), forms a nucleoprotein complex with the DNA and directs the 3′ single-stranded DNA tails to search out, invade, and pair with undamaged homologous sequences. DNA polymerases then carry out repair using the intact DNA as a template. The processes of DNA strand exchange and extension generate Holliday junctions, structures in which two double-stranded DNA duplexes are intertwined. The final step of the reaction is the resolution of the Holliday junction structures with specific nuclease enzymes [72]. Interestingly, several enzymes involved in NER also participate in different steps of the HR reaction. The proteins mentioned here form only a partial list, which is not intended to be complete, as there are several excellent reviews on the molecular events in HR.

Any defect in the double-strand breaks repair pathway is likely to lead to gross chromosomal rearrangements. There are indirect evidences that such repair systems might be affected in mouse liver with age. It is now well recognized that persistent double-strand breaks and gross chromosomal rearrangements increase in the liver of aged mice [15, 73]. The NEHJ function has been shown to decline with age in the brain of rats [74, 75]. Liver from old rats, however, does not show a decrease in Ku70, Ku80, DNA-PK_{CS} protein levels or activities compared to young rats [76]. There is little information for mouse liver in the literature. Based on microarray expression data, Hoeijmakers and colleagues [7] observed a twofold increase in mRNA levels for RAD51 and RAD51-like 1 proteins in the liver of 32-month-old mice compared to two-month-old animals. Han and colleagues [77] found RAD21 mRNA to be elevated in the liver of old mice. RAD21 is also involved in the repair of double-strand breaks [78]. Such increase in RAD51, RAD51-like 1, and RAD21 mRNA levels may reflect a response to the higher levels of double-strand breaks observed in hepatocytes of older mice [73]. One important limitation of microarray analyses is the absence of information on the actual protein levels in tissues under study. It is thus unknown at present whether the enzymatic activities related to HR (or to the NHEJ) in the mouse liver change with age.

5. Mitochondrial DNA in the Mouse Aging Liver

The mitochondrial theory of ageing is based on the assumption that ROS or other free radicals generated as byproducts of the mitochondrial electron transport chain during the life span of an organism damage proteins, lipids, and the mitochondrial DNA, which are closely positioned to the sites of ROS generation in the electron transport chain [25]. The mitochondrial genome is a circular molecule of approximately 16 s and codes only for components of the oxidative phosphorylation system (13 proteins in addition to 22 tRNAs and 2 rRNAs). A single hepatocyte may contain up to 25,000 copies of mitochondrial DNA (four to ten copies per mitochondrion), which are continuously turned over along with mitochondria, independently of the cell cycle [79, 80]. Oxidative damage is believed to play a substantial role in mitochondrial mutagenesis [45] because the majority of mitochondrial mutations are GC to AT transitions, which are signature mutations for oxidized cytosines [80]. Mitochondria possess a functional BER mechanism, some activities indicative of double-strand break repair [81], and recently mismatch repair activities have been identified in human cells and rat liver mitochondria [82, 83]. On the other hand, NER has not been detected in mitochondria [84]. The Cockayne Syndrome group B gene product (CSB), which is involved in NER and BER in the nucleus, has recently been found in human mitochondria, where it is believed to only assist BER reactions through protein interactions [85].

One study reported that most mitochondrial DNA in the liver of 22-month-old mice carry multiple point mutations, a significant increase compared to two-month-old animals. The mutation frequency in the liver of old mice ranged from 0.1 to 2.4%, which exceeds the mutation frequencies observed for nuclear DNA by approximately 1000-fold [86]. This observation is not, however, surprising, as the levels of oxidized DNA lesions are significantly higher in the mitochondrial DNA when compared to the nuclear DNA in rat and mouse liver [34, 87]. As in the nuclear DNA, oxidative DNA damage can lead to mutations but also to deletions. Indeed, the frequency of multiple deletions in mouse liver also increases significantly with age [88, 89]. The exact reason for the increase in deletions in the aging liver is yet unknown, but an important clue to the mechanism was given by the observation that the deleted regions in the mitochondrial DNA of old mice are flanked by small repeats [89]. Paradoxically, BER from mitochondrial extracts increases with age in the liver of mice and rats [59, 63], in contrast to data showing an increase in oxidative lesions in mitochondrial DNA with age [86]. This contradiction may have been resolved when it was found that although the total 8-oxoguanine-DNA glycosylase activity is higher in mitochondrial extracts from the liver of old mice, a large fraction of the enzymes is stuck to the membrane in the precursor form, which could not be translocated to and processed in the mitochondrial matrix. A similar phenomenon was observed with the mitochondrial uracil-DNA glycosylase responsible for the repair of mutagenic uracil in the DNA, suggesting a net decrease in repair with age [90].

DNA polymerase γ is the only known DNA polymerase in mitochondria, involved both replication and repair processes. This protein is encoded by a nuclear gene. Two groups have independently generated mice expressing a proof-reading-deficient DNA polymerase γ [91, 92]. These mice accumulate mitochondrial DNA mutations and deletions and exhibit a premature aging phenotype with a reduced life span. Interestingly, such mice do not exhibit increased markers of oxidative stress (including oxidized DNA damage) in the liver with age [92, 93]. The only age-related phenotype observed in the liver of proof-reading-deficient DNA polymerase γ mutant mice was extramedullary haematopoisis in the liver of six-month-old animals [91], while this phenotype generally occurs much later in normal mice [94]. It is believed that the accumulation of mitochondrial mutations in such mice is causing a decrease in functional proteins of mitochondrial electron transport chain [95]. This, in turn, impacted on energy production, cell proliferation and led to increased apoptosis in the liver tissue potentially affecting liver homoeostasis [91–93, 95]. Electron microscopical analyses of the mitochondria from the DNA polymerase γ mutant mice should give important information on the morphology and function of these organelles in the liver. Finally, the levels of telomeric rearrangement or attrition in such mice would also contribute to important information on the impact of mitochondrially generated ROS on telomere stability in aging mice.

6. DNA Repair Enzymes and Metabolic Syndromes

A major question that still remains is whether DNA repair deficiency leads to accumulation of enough mutations in the liver (or any other tissue) to cause aging or any age-associated diseases. Most DNA repair encoding genes have been mutated or deleted through genetic manipulation of embryonic stem cells to understand their functions and impact on aging. The goal of this section is to describe some of these mouse models of aging that we think are giving valuable information in the field of liver aging.

6.1. Base Excision Repair. The genetic ablation of XRCC1, APE/REF-1, β-pol, and DNA ligase III in mice is embryonic lethal [96–99], while the absence of 8-oxoguanine DNA glycosylase 1 (OGG1), endonuclease III homolog (mNTH1), or 8-oxodGTPase (MTH1) results in animals appearing normal, with no gross abnormality in the liver. They do accumulate premutagenic lesions, 8-oxoguanine DNA, and are prone to hepatocarcinoma in the presence of damaging agents (causing oxidative stress) [100–105], but no aging phenotype has been observed. PARP1-deficient mice, in turn, exhibit increased frequency of deletions and spontaneous liver tumors compared to wild-type animals [106]. In contrast, a knockout mouse model of the NEIL1 DNA glycosylase in mouse can result in a severe metabolic disorder [107]. Metabolic syndrome afflicts up to one half of Western population and is considered an age-related, pro-inflammatory lipidic disorder [108]. This phenotype, however, has not been reproduced in NEIL1-deficient mice from other laboratories and could be due to the genetic background of the mouse colony used in the published study [107]. Although OGG1 and NTH1 enzymatic reactions represent the bulk of DNA glycosylase activities in mammalian cells for repairing oxidized bases, NEIL1 is part of a back-up system for repairing ROS-induced lesions in mammalian mitochondria [38] and cells. Importantly, NEIL1 protein, unlike OGG1 and NTH1, is able to excise base lesions from single-strand DNA regions suggesting a preferential involvement of this enzyme in repair during transcription [109]. Whether a specific subset of genes involved in glucose and lipid metabolisms is more prone to the deleterious effect of NEIL1 loss of function in the liver of mice awaits further evidence.

6.2. Nucleotide Excision Repair. Two subpathways are recognized for the NER, which differ in the lesion recognition mechanisms. The global genome NER is involved in the removal of distorting lesions anywhere in the genome, while the transcription-coupled NER eliminates distorting DNA damage that blocks transcription [110]. As mentioned earlier, several mouse models with mutations in different NER genes were generated to phenocopy different progeroid syndromes [68]. These models include the XPA, CSB, ERCC1 knockout mice and the XPD point mutation animals [110]. Briefly, the XPA knockout mice are completely deficient for both global genome NER and transcription-coupled NER, but can repair some transcription-blocking lesions, including oxidative DNA damages. The CSB protein is thought to be involved in displacing RNA polymerase stalled by a DNA lesion and recruiting the NER (and probably the BER) machinery to sites of DNA lesions. CSB knockout mice are deficient in transcription-coupled NER and have lower BER activity of oxidative DNA damage, while their global genome NER capacity remains intact. Such mice show mild aging features including reduced growth and mild neurologic dysfunction [111]. Complete inactivation of the XPD helicase is not viable in mice, due to its essential transcription initiation function as a component of the Transcription Factor IIH complex. There is an XPD mouse model, however, that contains the same point mutation found in a trichothiodystrophy patient, which shows many hallmarks of trichothiodystrophy, including premature aging features [68]. This mutant XPD protein causes a partial defect in both global genome and transcription coupled NER. The ERCC1-XPF complex forms an endonuclease required for the 5′-incision to remove the damage-containing oligonucleotide during NER, but also essential for interstrand crosslink repair. ERCC1 knockout mice are deficient in global genome NER, transcription-coupled NER, and interstrand crosslink repair. These mice show a severe phenotype including retarded growth, progressive neurological abnormalities, kyphosis, a short life span of about three to four weeks, and liver dysfunction [112]. At the cellular level, an ERCC1 defect leads to accelerated nuclear polyploidization. The combination of a knockout allele with a truncated ERCC1 allele, resulting in a protein lacking the last seven amino

acids, delays the onset of the premature aging phenotype and extends the maximal life span to about six months [113].

XPA, CSB, ERCC1 knockout mice and the mutant XPD animals were crossed to mice harboring *lacZ*-reporter genes, and mutation frequency was estimated in the liver of these mice by Dollé and colleagues [110]. XPA deficiency resulted mainly in one-base pair deletions in young mice, but in older mice the mutation spectrum changed to an increase in G:C → T:A transversions, which are characteristic of oxidative DNA damage, indicating that an increase in oxidative DNA damage is an important event in age-related mutagenesis in the liver. ERCC1 mutant mice (expressing the truncated protein), with their short life span of six months and severe symptoms of premature aging, displayed an even faster *lacZ*-mutant accumulation in the liver. Mutations included mostly genome rearrangements [110]. In contrast, CSB knockout mice and the XPD point mutation animals did not reveal an elevated *lacZ* mutation frequency. The authors concluded that shortened life span in mice with defects in transcription-coupled repair did not depend upon increased mutation accumulation [110]. Interestingly, global gene expression analyses using microarrays revealed that the same mutated XPD mice exhibited increased apoptosis that exceeded cell renewal in their liver [114]. Appropriate histochemical analyses confirmed the microarray data. In addition, these mice displayed major metabolic changes with regards to lipid metabolism (abnormal lipofuscin accumulation in hepatocytes) and a downregulation of the Insulin Growth Factor 1 (a known regulator of life span) signaling in their liver [114, 115]. In the case of ERCC1 knockout mice, metabolic and gene expression profiling also revealed altered lipid and energy metabolism, transition to ketosis, increased liver cell death, and attenuation of liver functions [7, 116]. Finally, these mice also displayed downregulation of the Insulin-like Growth Factor 1 signaling in their liver [116].

A double knockout of XPA and CSB genes in mice resulted in a complete inactivation of NER leading to a phenotype that reliably phenocopied the human progeroid Cockayne syndrome [117]. Mouse liver transcriptome analysis and several physiological endpoints revealed systemic suppression of the growth hormone/insulin-like growth factor 1 somatotroph axis, changes in oxidative metabolism with increased antioxidant responses, and hypoglycemia together with hepatic glycogen and fat accumulation [117]. Importantly, wild-type mice exposed to a low dose of chronic genotoxic stress recapitulated this response. Altogether, these results suggest that even though mutation rates may not be tremendously high in these mice, impaired transcription of genes containing DNA lesions would still contribute to aging due to the intimate relationship between NER and transcription in the liver of NER-deficient mice [69].

6.3. Double-Strand Break Repair. There is a lot more information regarding mice deficient in proteins of the NHEJ repair pathway and aging liver than those lacking proteins of the HR pathway [69, 71]. DNA-PK_{CS}-deficient mice develop hepatitis with very high frequencies (66% of all mutant mice examined) [118], although no other liver anomalies were reported. Ku80-deficient mice exhibit signs of premature aging in several organs, and their livers displayed micro abscesses (acute response with neutrophils) and granolamatous inflammation (chronic response with mononuclear cells), as well as nodular hyperplasia [119]. Moreover, Ku80-deficient mice harboring the *lacZ*-reporter gene displayed significantly higher numbers of genome rearrangements in liver compared to wild type animals [120]. The liver of such animals also exhibited substantial increase in persistent γ-H2AX DNA damage foci as compared to wild-type liver. On the other hand, little information has been published on the liver of Ku70-deficient mice, and only one report suggests that Ku70-deficient mice treated with an alkylating agent will rapidly develop hepatocellular carcinomas [121].

The WRN protein is a member of the RecQ helicase family that also contains a functional 3′-5′ exonuclease activity. It is involved not only in the NEHJ repair pathway but also in HR, in telomere maintenance, and potentially in BER pathways [122]. Complete functional inactivation of the WRN protein in human leads to the premature aging disorder Werner syndrome. The first mouse WRN mutant model to be described in the literature lacked part of its DNA helicase domain [123]. These mice synthesize a stable protein with no DNA helicase activity. Originally, these WRN mutant mice (largely in the outbred Black Swiss genetic background) showed almost no phenotype after one year of age, prompting several researchers to infer that these mice did not age prematurely [71]. This conclusion was reinforced by the description of a WRN null mouse that showed no age-associated phenotype [124]; these animals would show metabolic anomalies, including diffuse fatty infiltration of the liver, only if fed a diabetogenic diet [125]. However, WRN null mice crossed into telomerase knockout background exhibited metabolic anomalies and severe premature aging concomitantly with telomere attrition after several generations, stressing the importance of WRN functions at the telomeres [126, 127].

Realizing that the genetic background may have profound impact on the phenotype of mutant mice, the Lebel laboratory backcrossed the WRN helicase mutant outbred mice onto the pure C57BL/6N strain for twelve generations. These C57BL/6N WRN helicase mutant mice did not severely age prematurely, but displayed phenotypes associated with human Werner syndrome. They exhibit an abnormal hyaluronic acid excretion, premature visceral obesity, hypertriglyceridemia, insulin-resistant type 2 diabetes and associated cardiovascular diseases, higher ROS levels in several tissues, increased genomic instability, and cancer incidence resulting in a 10–15% decreased mean life span [128]. Importantly, WRN helicase mutant mice exhibit a severe defenestration of the liver sinusoidal endothelial cells at seven to nine months of age [129], which is not observed in wild-type animals until the age of two years. Interestingly, this morphological change is reversed by vitamin C or resveratrol treatments [129, 130], indicating a causative role for oxidative stress. In addition to morphological changes, WRN helicase mutant mice exhibit liver steatosis, increased lipid

peroxidation, and oxidative DNA damage. Furthermore, these mutant mice displayed increased phosphorylation activities of kinases normally responding to oxidative stress [129]. Liver tissue from WRN helicase mutant mice also exhibited alteration in the expression of genes involved in caloric restriction [131], glutathione and xenobiotic metabolism by cytochrome P450 pathways. These results can be interpreted as a transcriptional response to the elevated oxidative stress in the mutant mice. These mutant mice also showed increased expression of genes involved in inflammation, indicating an inflammatory response in their liver [129, 130]. The observed alteration of the global gene expression profile in the liver of these mutant mice suggests that WRN protein may affect promoters of specific subset of genes directly [132] or indirectly. Finally, WRN helicase mutant mice exhibit increased rate of point mutations in liver mitochondrial DNA [129, 130]. Preliminary electron microscopy of liver tissue from helicase deficient WRN mutant mice has indicated a decrease in the number of mitochondria and altered morphology of these organelles compared to liver cells from age-matched wild-type animals [133], a phenotype reminiscent of ageing liver [134–136]. Concomitantly, WRN helicase mutant mice exhibited a decrease in ATP production [129]. Additional experiments will be required to determine whether the mitochondrial dysfunction is secondary to the dyslipidemia or diabetes seen in these mice [128, 129] or if it constitutes a primary event, which could be responsible for the premature aging phenotype.

7. Conclusions

Aging is a pleiotropic and stochastic complex process that is heavily influenced by genetic, epigenetic, and environmental factors. This is particularly relevant for the liver, which is a major organ regulating whole body homeostasis. The liver is central to glucose and lipid metabolism as well as steroid hormone biosynthesis and degradation. Importantly, it is the main detoxifying organ with regards to drugs or potentially toxic chemicals found in food. It has become clear, using mouse models as biological tools, that genetic instability in the form of gross DNA rearrangements or point mutations accumulate in the liver with age. DNA lesions, such as oxidized bases or persistent breaks, also increase with age and correlate well with the presence of senescent hepatocytes. The level of DNA damage and/or mutation can be affected by changes in carcinogen activation (during detoxification steps of procarcinogenic substances), decreased ability to repair DNA, or a combination of these factors [52]. ROS tissue level (exogenous or endogenous generated during normal metabolism) impacts on liver functions, and is intimately linked to most if not all age-associated diseases. Mitochondrial dysfunction is one major source of endogenous ROS during aging. Whether increased ROS is causative or a consequence of aging is still a subject of intense debate, especially in light of the observation that mitochondrial DNA polymerase γ mutant mice age prematurely without increasing ROS levels [92, 93] in different tissues including the liver. It is clear, however, that mitochondrial dysfunction due to accumulation of either mitochondrial DNA deletions or point mutations will affect energy balance in cells and, by the same token, the renewal of the stem cell compartment of a tissue or simply by altering highly metabolically active cells like the hepatocytes.

Telomere attrition is also associated with aging, although similarly to what was discussed for ROS, whether telomere shortening is causative or a consequence of aging is still a subject of debate. Considering the fact that the telomeric sequence is G rich, increased ROS will affect binding of telomere specific proteins and potentially induce a telomeric-dependent senescence pathway in cells, which again will impinge on liver homeostasis at the cellular level. In this context, telomeres would act as an "ROS sensor" in the cell gauging the levels of DNA damage in the liver. Causative or not, ROS levels and structural changes at the telomere will likely exacerbate the aging phenotype or dysfunction of a tissue.

The *lacI/lacZ* reporter gene mouse models have taught us that different tissues exhibit different mutation rates with age. Specific DNA repair pathways have been shown to decline with age, depending on the tissues. Except for the BER pathway, few studies have shown decline of other DNA repair pathways or repair enzymes in the mouse aging liver. As several DNA repair enzymes are posttranslationally modified upon DNA damage (thus altering their activities), appropriate experiments are warranted to follow such post-translational changes at the protein levels in the liver of aging mice. Noteworthy, the genetic background of the mice under study and the husbandry conditions (including diet) will also impact on the phenotypes. Thus, depending on the stress imposed on mice, the severity of the phenotype will vary. Nevertheless, the control of ROS levels, structural changes at the telomere, DNA damage and mutation rate, mitochondrial dysfunction will ultimately impact on health, and such processes underline the complexity of aging.

It remains unclear why only certain DNA repair mutants show phenotypes related to premature aging. It is interesting to note that the DNA repair-deficient mouse models that exhibit reduced health and/or life span in addition to early appearance of age-related phenotypes also display major changes in the expression of liver genes involved in stress response, cell proliferation and apoptosis, glucose and/or lipid metabolism, and inflammatory response. This suggests that NEIL1 (associated with BER), CSB, ERCC1, XPA, XPD (associated with NER), DNA-PKcs/Ku complex (associated with NHEJ), and WRN (associated with NHEJ, HR, or BER) are also implicated (directly or indirectly) with the transcription of a subset of genes (or pathways) important for the aging phenotypes at least in the liver. Such data imply the possibility of targeting specific biochemical pathways (in addition to ROS levels, telomere structural changes, mitochondrial dysfunction) to control or slow down the progression of age-related diseases. The impact of calorie restriction, dietary restriction mimetics, or antioxidants is already under scrutiny in different mouse models of aging [129, 130, 137, 138].

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