Pathogenesis of chronic pancreatitis

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ABSTRACT: Studies from the Marseille group allowed differentiation of acute pancreatitis (a group of lesions secondary to either extrapancreatic causes such as gallstones or to pancreatic diseases such as cancer and chronic pancreatitis), from chronic pancreatitis. Two forms of chronic pancreatitis are easily distinguished: obstructive pancreatitis secondary to pre-existing obstruction on pancreatic ducts (tumours, scars, etc); and the most frequent disease, chronic calcifying pancreatitis, which is a pancreatic lithiasis due to a double phenomenon. This double phenomenon is the precipitation of insoluble calcium salts and the precipitation of degraded fragments of a newly discovered secretory protein known as pancreatic stone protein (PSP). This family of glycoproteins, the amino acid sequence of which has been established, is synthesized by the pancreatic acinar cell and its biosynthesis is decreased in patients presenting with chronic calcifying pancreatitis. The secretory form of PSP prevents the formation of calcium salt crystals in the pancreatic juice which is normally supersaturated in calcium. Though the lesions and the secretory modifications of PSP are common to all forms of chronic calcifying pancreatitis, there are different etiological forms of the disease: alcoholic, tropical, hypercalcemic, hereditary and idiopathic. Alcohol consumption acts on pancreatic secretion by different mechanisms and is responsible for an increased secretion of secretory protein (enzymes) due to cholinergic, vagal reflexes sensitive to ethanol. Alcohol consumption is generally associated with protein rich and either fat rich or fat poor diets, both of which are risk factors. Hypercalcemia also increases the secretion of protein and the viscosity of pancreatic juice. The tropical form is not due, as previously suggested, to cassava (manioc) consumption or kwashiorkor, but it is endemic in populations submitted to fat poor, protein poor diets. These etiological factors are only acting on predisposed patients. This suggests that a low or abnormal biosynthesis of PSP is responsible for the predisposition. Can J Gastroenterol 1989;3(1):15-20 (Pour résumé, voir page 16)

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Acute and chronic pancreatitis are considered by some authors as different stages of the same disease, the recurrence of acute pancreatitis being the origin of chronic pancreatitis (1). Studies have shown, however, that acute lesions healed without sequelae after a short stage of reversible fibrosis (2-5). Furthermore, the age of patients presenting with acute pancreatitis, the presumed cause, is 13 years older than that of patients with chronic pancreatitis, the presumed consequence, and etiological data of both groups of patients are significantly different (6). This has been the basis of the two Marseille classifications (7,8) which distinguish acute and chronic pancreatitis as has already been done in classical books (9).

Acute pancreatitis is not a disease but a group of anatomical lesions: edema, necrosis, hemorrhagic necrosis and fatty necrosis. It is frequently due to an extrapancreatic cause such as gallstones but is often a complication of chronic pancreatic diseases, such as pancreatic cancer or chronic pancreatitis. When the cause of these lesions persists, acute pancreatitis may recur but will heal without sequelae if its cause, for instance gallstones, is treated with success.
RESUME: Les travaux du groupe de Marseille ont permis d'individualiser: des pancréatites aiguës qui sont un groupe de lésions secondaires à des causes soit extrapancréatiques (par exemple la lithiase biliaire) soit intrapancréatiques (compilant par exemple les pancréatites chroniques); des pancréatites chroniques qui sont un groupe de maladies au sein duquel on peut déjà séparer des pancréatites obstructives dûes à un obstacle sur les voies pancréatiques pré-existant à la maladie (cancer, cicatrice, etc) et des pancréatites chroniques calcifiantes, de beaucoup les plus fréquentes. Celles-ci sont en réalité une lithiase pancréatique due à un double phénomène, d'abord microscopique puis macroscopique: précipitation de carbonate de calcium et précipitation d'un fragment dégradé d'une molécule sécrétée nouvellement découverte, la PSP (protéine stabilisatrice du pancréas). Cette famille de glycoprotéines dont la séquence en aminoséquences est actuellement connue est synthétisée par la cellule acinuse du pancréas et sa synthèse diminuée dans les pancréatites chroniques calcifiantes. La forme sécrétoire de PSP prévient la formation de cristaux de sels de calcium dans le suc pancréatique et est normalement sursaturé en calcium. Bien que les lésions et les modifications de la PSP soient semblables dans toutes les formes de pancréatite calcifiante, il existe plusieurs étiologies: alcoolique, tropicale, hypercalcémique, héritaire et idiopathique. L'alcool agit sur la sécrétion pancréatique par des mécanismes divers, essentiellement une hypersécrétion de protéines (enzymes) due à un réflexe long cholinoergique empruntant le nerf vague. L'alcoolisme est généralement associé à des régimes hyperprotidiques et soit hyper-, soit hypolipidiques qui sont autant de facteurs de risque. L'hypercalcémie augmente également la sécrétion de protéines enzymatiques. La forme tropicale ne parait être liée ni à la consommation de manioc ou cassava, ni au kwashiorkor mais s'observe dans des zones où l'ensemble de la population est soumise à un régime pauvre en protides et en lipides. Ces facteurs étiologiques n'agissent que sur des sujets prédisposés. Il est possible que la prédisposition soit une baisse de la biosynthèse ou une synthèse anormale de PSP.

Chronic calcifying pancreatitis is characterized by persisting destruction of pancreatic parenchyma replaced by fibrosis. Whatever their type, and particularly during the first years of its evolution, it is frequently complicated by bouts of acute pancreatitis which progressively regress when the exocrine pancreatic insufficiency is installed (10-12).

It is now possible to distinguish different forms of chronic pancreatitis presenting with different and specific lesions and etiologies (13-16).

Obstructive pancreatitis: Obstructive pancreatitis is due to the occlusion of the main pancreatic duct, or of one of its branches, before the area of pancreatitis, for example, tumours, fibrous scars of necrotic pseudocysts, or congenital anomalies. The lesions are regularly sprayed in the occluded territories, the duct epithelium is relatively preserved, pancreatic calculi are not found and protein precipitates are rare.

Calcifying chronic pancreatitis (CCP): CCP is by far the most frequent form of chronic pancreatitis, representing more than 95% of cases. Though CCP is due to different causes (alcoholism, tropical juvenile form, hereditary, hypercalcemia, idiopathic), its lesions are similar regardless of etiology (13, 15, 16). They are characterized by their patchy distribution: the intensity of the lesions of one lobule or group of lobules (and of the duct draining them) is different from neighbouring lobules, varying from a completely normal appearance to complete destruction. Duct lesions are important: atrophy of epithelium, scars with formation of strictures and of retention cysts and deformation of the duct. The ducts contain protein plugs which will later calcify to form pancreatic calculi generally visible on X-ray films of the abdomen, after some years of evolution (10-12), perineural inflammatory infiltration in fibrosis.

A morphometric ultrastructural study of the acinus and duct cells (17) has shown that the first lesion of CCP, before lesions of the duct and acinar cells, was the formation in the ducts of eosinophilic protein plugs. All transitions exist between these precipitates and calculi (commonly called calcifications) (15,18-20).

Chronic calcifying pancreatitis, the most frequent chronic inflammatory disease of the pancreas is, therefore, a lithiasis which more resembles kidney lithiasis than alcoholic liver cirrhosis. As this disease has different causes and similar lesions, there must be some mechanisms (common to all etiological forms, and others particular to each etiology.

MECHANISMS COMMON TO DIFFERENT FORMS OF CCP

Protein plugs at the origin of the disease are a build up of a fibrillar proteinaceous material (20,21). Mature calculi contain calcium salts, mostly bicarbonate (calcite) and a small quantity of protein (18-20,22). Morphological studies suggest that calculi originate from protein plugs by the deposition of calcite and a network of protein fibrils (18,19). The protein material of plugs (23) and of calculi (22) is similar. This low molecular weight protein, pancreatic stone protein (PSP), isolated in the authors' laboratory is associated in calculi with an unidentified polysaccharide (22). The authors have shown that this composition was identical in the alcoholic, idiopathic and in the Indian juvenile tropical forms (24). In most cases, calcium salts represent at least 95% of weight and are associated with small quantities of a soluble form of PSP. Nevertheless, giant calculi (2 cm) composed of pure calcite without PSP were observed in a nonalcoholic woman (25). Inversely, some rare calculi are composed only of an insoluble, probably degraded, molecular form of PSP which may later calcify. The pathogenesis of CCP raises, therefore, two problems: precipitation of calcium and precipitation of protein. Both problems are dominated by PSP.

Precipitation of calcium: Pancreatic juice is always saturated in calcium (26). This means that the real problem is to determine why calcium does not precipitate in normal subjects as it precipitates in patients presenting with CCP. It is necessary to assume the existence of one or more stabilizers in the normal pancreas.
atic juice, preventing the precipitation of calcium when it is saturated.

PSP is probably one of these stabilizers. This molecule is present in normal pancreatic juice as a group of proteins, molecular weight 15,000 to 19,000 (27). The molecule which is secreted by acinar cells is probably the heaviest one; it is glycosylated. It is synthesized as a unique amino acid chain in the endoplasmic reticulum of the acinar cell, which contains a specific messenger RNA for PSP (28). It is concentrated as enzymes in zymogen granules (29).

In pancreatic juice, the four heaviest molecular forms (PSP-S2 to S5) are hydrolyzed by trypsin (30) to a smaller form (PSP-S1), insoluble at physiological pH, nonactive in preventing calcium crystallization. The complete amino acid sequence of this form has been published (31). It is a 133 amino acid peptide and its sequence has no homology with the sequence of any other known protein, particularly pancreatic enzymes (27). PSP extracted from calculi probably has the same amino acid chain as PSP-S1, but is soluble at physiological pH and prevents calcium crystallization. When a calcium chloride solution is added to a solution of sodium bicarbonate and different ions, (the concentration of which mimics the ionic composition of pancreatic juice), after a 2 to 3 min delay (nucleation time) a precipitate of calcium chloride (calcite) is formed. If adequate quantities of PSP are added before calcium chloride, no crystal precipitates are formed. When PSP is added after precipitation has started but before it is complete, the process stops immediately. Similar molecules preventing crystallization of calcium salts are already known in saliva (32) and urine (33). They act by blocking the growth sites of crystals, which indicates that one molecular form of PSP is found in almost pure conditions in pancreatic calcified calculi.

PSP exists in the pancreatic juice of all mammals studied in the authors' laboratory. A degraded form, insoluble at pH 7, has been discovered in the ox pancreas and in the human pancreas by Gross and co-workers (34,35). When PSP is measured in pure pancreatic juice by immunological techniques using polyclonal antibodies (Mancini immunodiffusion [36], Elisa with a monoclonal antibody as first antibody and a polyclonal antibody as the second antibody [37]), its concentration is constantly decreased in patients presenting with CCP compared to normal controls. This suggests that a decrease of PSP secretion could be responsible for the formation of calcic calculi.

It is not known if this decrease is due to a congenital disturbance of the biosynthesis of PSP (the case of complete absence of PSP is probably due to this mechanism [25]), or to the biosynthesis of an abnormal molecule (abnormal sequence of amino acids or abnormal glycoylatation). An in vivo degradation of PSP in pancreatic juice is improbable as PSP is strongly decreased in the zymogen granules of patients presenting with CCP compared to controls (29).

As soon as the duct epithelium is in contact with plugs or stones, the basal membrane disappears (38) and then the duct cell atrophies (15). This allows the transudation of interstitial fluid, which increases the concentration of serum protein (39) and calcium (40) of pancreatic juice. This increased concentration of calcium, secondary to the first lesions of the disease, could explain why calculi frequently appear and grow at the end of years of clinical evolution (10,11).

Precipitation of protein: Morphometric studies (17) have shown that the first visible lesion of the disease is the precipitation of protein. Biochemical studies (23) have shown that these proteins were formed by a degraded molecule insoluble at pH 7, corresponding to PSP-S1.

The assumption of the pathological secretion by acinar cells of an abnormal molecular form, less active and less soluble, could explain this fact, though this has not been demonstrated. It is, nevertheless, compatible with the finding that, if immunological estimation of PSP with polyclonal antibodies easily distinguishes patients presenting with CCP from normal patients (36,37), immunological methods using a monoclonal antibody prepared in the authors' laboratory do not show differences between patients and controls (37,41). This monoclonal antibody recognizes PSP-S1, the short, insoluble and inactive form of PSP.

ETIOLOGICAL FORMS AND GEOGRAPHICAL DISTRIBUTION

There are two areas of maximum frequency. In temperate countries, Europe, temperate zone of America, Africa, Japan (42,43), the disease is most frequently observed in alcoholics having a high protein and fat consumption, especially males. Generally, the clinical evolution starts between 30 and 45 years of age. When the consumption of alcohol, protein and fat increases with time in an ocidental country, with a delay of some years, an increased frequency of CCP is observed.

In certain tropical countries, CCP is observed in young adults (mean age 12 years) and nonalcoholics living in areas where protein and fat intakes are low (43), such as south India (45,46). This tropical form, or fibrocalculous pancreatic diabetes (46), is also observed in certain countries of Africa, such as Zaire (47) and Nigeria (48,49), but it is absent or exceptional in Senegal (50), Ivory Coast (51), Uganda (52) and Natal (53), where the origin is generally alcoholic. Some cases have been described in Brazil where the alcoholic form predominates (54).

CCP is observed in 5 to 7% of patients presenting with hypercalcemia secondary to hyperparathyroidism (55,56) or other causes (57). There is an autosomal hereditary dominant form with variable penetrance observed in children of both sexes (58,59). But also, more frequently than if by chance, one or two cases are observed at the adult age in the family of a patient presenting with CCP (6). Above, the authors have described exceptional forms: calculi of pure calcite without PSP and transparent calculi of degraded PSP without calcium. These forms are generally observed in young nonalcoholic women. It is, therefore, probable that CCP is, in reality, a group of different diseases.

ALCOHOLIC CCP

Anatomical form of alcoholic CCP: Two forms have been described: chronic pancreatitis, which is a chronic calcifying pancreatitis; and acute pancreatitis. One generally accepts that acute alcoholic pancreatitis is the exacerbation of the initial stages of chronic alcoholic pan-
creatitis. It is observed in chronic alcoholics who have recently increased their consumption of alcohol (60). The follow-up of these patients generally shows that they will, after some years, develop pancreatic calcification (61). A recent study concluded that certain cases of acute alcoholic pancreatitis healed completely and were not followed by chronic pancreatitis (62). In the authors’ opinion, this work proves only that the clinical onset of chronic pancreatitis appears at variable speeds after beginning alcohol consumption and does not allow either acceptance or rejection of the existence of acute alcoholic pancreatitis healing completely.

Alcohol, protein and fat consumption and risk of CCP: The authors have shown that there is a linear relationship between the logarithm of the risk of developing CCP and the average daily consumption of ethanol (63,64). Risk in total abstainers is lower than in subjects consuming such low quantities as 1 to 20 g ethanol per day. This means that there is no statistical threshold of tolerance but rather a continuous spectrum of individual thresholds from idiopathic pancreatitis to pancreatitis observed in heavy alcoholics, and that only some people are able to develop an alcoholic pancreatitis and, therefore, that there must be a congenital or acquired predisposition to the disease. Duration of consumption of ethanol is also important.

There is also a linear relationship between protein intake and logarithm of the risk, but the effect of protein is weaker than that of ethanol. The effect of fat is more complex: lower risk for average diets (80 to 100 g fat per day) which should, therefore, be prescribed to patients with an increased risk. The action of protein, fat and ethanol are additive on the logarithm of the risk. Tobacco also probably increases the risk of developing CCP (65,66).

Modifications of pancreatic juice due to alcohol: Chronic alcoholism increases total concentration of protein (mostly secretory enzymes), decreases pH, bicarbonate and citrate as well as the concentration of the secretory trypsin inhibitor and the ratio of cationic trypsinogen to anionic trypsinogen (67,68).

Mechanisms of action of ethanol: Alcohol is metabolized by the pancreas (69) but there are no arguments showing that this metabolism could play a role in the formation of lesions; alcoholic pancreatitis is a lithiasis. Similarly, there is no actual proof showing that the action on gastric secretion, tone of the sphincter of Oddi, or release of hormonal peptides or acetylmethyde produced by ethanol metabolism, could play a role (67,68).

On the contrary, in the dog and very probably in humans, acute alcohol consumption inhibits or stimulates pancreatic protein secretion according to its plasma concentration. This is produced by reflex mechanisms, the vagus nerve and nicotinic and muscarinic synapses being necessary (70). In cases of long duration (months or years) or alcohol consumption, the inhibitory reflex disappears which is responsible for an increased secretion of protein, very probably due to an increased cholinergic tone (67,68,71). This increases the viscosity of pancreatic juice and creates an obstacle to the flow of juice.

TROPICAL CCP

Epidemiological studies allow the comparison of the frequency of the disease as a function of diets in tropical countries (67,68). These studies are not favourable to a possible role of kwashiorkor, nor of the consumption of manioc or cassava which have been suggested. The only factors which seems to be statistically related to the disease are birth and life in areas of malnutrition and a very low fat diet. Low protein diet, if it plays a role, does not seem to be sufficient. A recent assumption based on rat experiments that protein deficiency of the mother is responsible for an increased secretion of protein, continuing for a long time after weaning (72).

Besides tropical calcifying pancreatitis, one finds in certain tropical countries a completely latent pancreatic insufficiency which probably affects all of the population (73).

HYPERCALCEMIC CCP

The pancreatic juice of patients presenting with hypercalcemia, for example that of alcoholics, has an abnormally high concentration of protein (74,75). The mechanism of this protein hypersecretion is complex: increased release of cholecystokinin (76); decreased sensitivity of duct cells to secretin (77); increased sensitivity of acinar cells to cholinomimetics and cholecystokinin (78).

PREDISPOSITION FACTORS

Not all alcoholics or all subjects submitted to hypercalcemia or living in tropical areas develop pancreatitis. It is, therefore, necessary to assume a predisposition. It is probable that the risk of another case in the family is greater for patients presenting with a CCP than in the general population (79). A higher risk is associated with blood group O (80,81); however, data on the HLA groups are contradictory (68).

The knowledge of chronic pancreatitis has progressed considerably over the years. Distinction from acute pancreatitis, the evolution of which is different even if it recurs; definition of different forms of chronic pancreatitis presenting with different lesions and causes; demonstration of nutritional risk factors and overall new molecular basis, indicate that this knowledge will continue to progress at accelerated speed in the following months and years.

REFERENCES

30. Harada H, Ueda O, Yasuoka M. Scanning electron microscopic studies

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20. Harada H, Ueda O, Yasuoka M. Scanning electron microscopic studies

20. Harada H, Ueda O, Yasuoka M. Scanning electron microscopic studies

47. Sonnet J, Brisko P, Bastin JP. Chronic