Research Article

A Unifying Hypothesis for Familial and Sporadic Alzheimer's Disease

Carole J. Proctor[1] and Douglas A. Gray[1, 2, 3]

[1] *Centre for Integrated Systems Biology of Ageing and Nutrition, Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne NE4 5PL, UK*
[2] *Ottawa Hospital Research Institute, Ottawa, ON, Canada K1H 8L6*
[3] *Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON, Canada K1H 8M5*

Correspondence should be addressed to Carole J. Proctor, carole.proctor@ncl.ac.uk

Alzheimer's disease (AD) is characterised by the aggregation of two quite different proteins, namely, amyloid-beta (Aβ), which forms extracellular plaques, and tau, the main component of cytoplasmic neurofibrillary tangles. The amyloid hypothesis proposes that Aβ plaques precede tangle formation but there is still much controversy concerning the order of events and the linkage between Aβ and tau alterations is still unknown. Mathematical modelling has become an essential tool for generating and evaluating hypotheses involving complex systems. We have therefore used this approach to discover the most probable pathway linking Aβ and tau. The model supports a complex pathway linking Aβ and tau via GSK3β, p53, and oxidative stress. Importantly, the pathway contains a cycle with multiple points of entry. It is this property of the pathway which enables the model to be consistent with both the amyloid hypothesis for familial AD and a more complex pathway for sporadic forms.

1. Introduction

Alzheimer's disease (AD) is characterised by the presence of extracellular amyloid-beta (Aβ) plaques and cytoplasmic tau tangles and the loss of neurons in specific regions of the brain. The connection between these events is still not clear although it has been proposed that the formation of plaques precedes the appearance of tangles which in turn precedes cell death [1, 2]. Confounding the acceptance of such a simple temporal order of events is evidence that plaques are not necessary for disease progression [3] and that the accumulation of plaques can also occur as part of normal ageing with no apparent pathology [4]. Moreover, soluble Aβ may be a better correlate of disease than the insoluble plaques [5, 6]. It has recently been suggested that the amyloid hypothesis may only hold for familial forms of the disease but that the situation is much more complex in late-onset forms [7]. It is also possible that Aβ is a damage response protein [8]. Small and Duff [7] suggest that the pathway between Aβ and tau is linear for early-onset AD but hypothesize that a dual pathway links the two in late-onset disease [7]. A number of molecular pathways have

been proposed as the upstream driver of both Aβ and tau aggregates. One important candidate is glycogen synthase kinase-3β (GSK3β). It is well established that GSK3β activity leads to hyperphosphorylation of tau and there is also evidence that it accounts for increased production of Aβ [9]. Its importance in AD was highlighted in 2008 by the proposal of a "GSK3 hypothesis of AD" [10]. A recent review also surveys data in support of the contention that GSK3β provides the link between Aβ and tau [11]. In addition it has been shown that Aβ behaves like an antagonist of insulin and prevents activation of Akt [12]. Akt phosphorylates GSK3β which inhibits its activity; Aβ therefore indirectly increases the activity of GSK3β. There is also a link between p53 and GSK3β and we recently modelled this to show that this interaction might explain the link between protein aggregation and neuronal loss in AD [13]. The model predicts that GSK3β overactivity leads to an increase in levels of Aβ plaques and tau tangles by independent processes supporting the idea of a dual pathway.

One way to examine the order of events in disease pathology is to prevent the formation of plaques and then observe whether or not tau tangles appear. An experimental
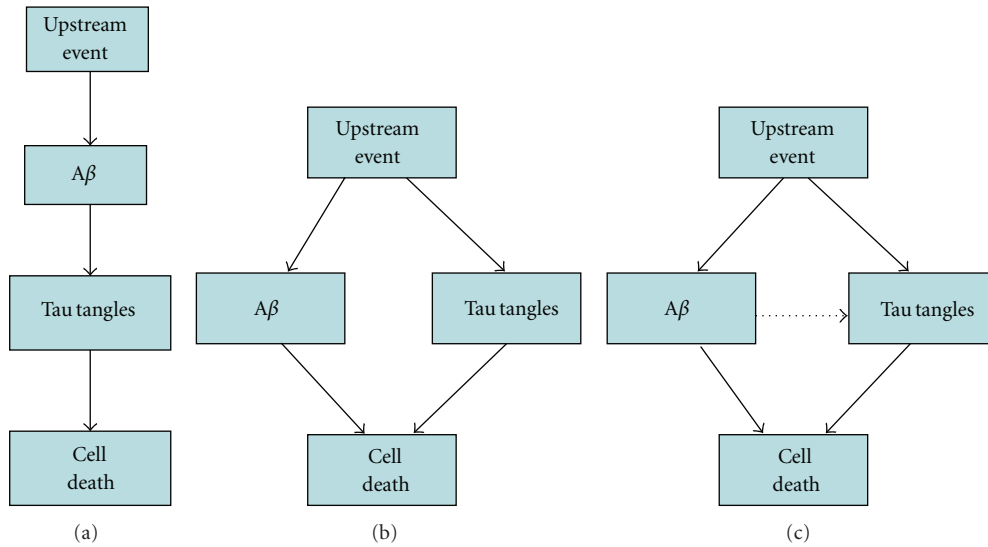
FIGURE 1: Alternative hypotheses for the link between Aβ and tau. (a) Linear pathway. (b) Dual pathway. (c) Complex pathway.

procedure for doing this is Aβ immunization which has been carried out in many mouse models and also in a number of human clinical trials. Many of the mouse models do not have tau pathology and so cannot be used to test hypotheses concerning the order of events. In the more relevant 3×Tg-AD mouse model, experiments indicated that reducing plaques also led to the clearance of early tau pathology [14]. On the other hand human clinical trials have not shown any clear evidence of a reduction in tau tangles in regions where plaques were reduced [15]. Our model of GSK3/p53 [13] can examine the effect of increased clearance of Aβ by the simple modification of increasing the rate of Aβ (soluble form) removal. By doing so it is possible to test whether there is a linear or a dual pathway. If the pathway is linear, then the model should predict that increasing clearance of Aβ will also reduce the formation of tau tangles (Figure 1(a)). If there is a dual pathway, then increasing the clearance of Aβ will not affect the levels of tau tangles (Figure 1(b)). However, there is a third possibility (complex pathway): Aβ may not directly affect the formation of tau tangles in a linear pathway but may still have indirect effects (Figure 1(c)).

2. Methods

We previously built a stochastic dynamic model of p53 regulation [16] which was then extended to include GSK3β, Aβ and tau [13]. The models are encoded in the Systems Biology Markup Language (SBML), a computer-readable format for network models [17]. SBML allows models to be easily modified and extended and also enables sharing of models since the code is publicly available from the Biomodels database [18]. The extended GSK3 model includes a module for the DNA damage response which leads to elevated levels of p53, which can then bind to GSK3β. We assume that binding of GSK3β to p53 increased the activity of both proteins. The model includes a module for p53 turnover in which we assume that p53 binds to the E3 ligase Mdm2

and is then ubiquitinated and targeted for degradation by the 26S proteasome. Under normal (unstressed) conditions, both p53 and Mdm2 are kept at low basal levels. However, when cells are stressed and DNA damage occurs, p53 is phosphorylated and is then unable to bind to Mdm2 and so is no longer degraded. Therefore p53 levels increase. In addition, phosphorylation of p53 increases its activity. Full details of this module are already published [16]. Under normal conditions when p53 levels are low, it is unable to bind to GSK3β and so we assume that GSK3β activity is low when cells are not stressed.

The model also includes reactions for the production, clearance and aggregation of Aβ, and the phosphorylation/dephosphorylation and aggregation of tau. In addition we assume that Aβ results in increased generation of ROS and increased transcription of p53. The full details of the model are available in an open access journal and the SBML code is available from Biomodels (BioModels ID:BIOMD0000000286)[18]. The simulations were carried out using the Gillespie algorithm on the Biology of Ageing e-Science Integration and Simulation (BASIS) system [19–21]. The model results were analysed and plotted using the R package.

3. Results

3.1. Increased Aβ Clearance from Day 0. In our previous model we set the rates for aggregation of tau and Aβ at levels so that if there was an increase in tau phosphorylation or an increase in Aβ production, the formation of aggregates would appear within 2 or 3 days. In reality, the aggregation process is likely to have much longer lag periods. Acceleration of the aggregation process in our computer model is merely a device to increase the throughput of simulations. With normal rates of Aβ clearance, our model predicts that a small percentage of cells do not accumulate any plaques or tangles by 12 days (Figures 2(c) and 2(f)). However, the majority
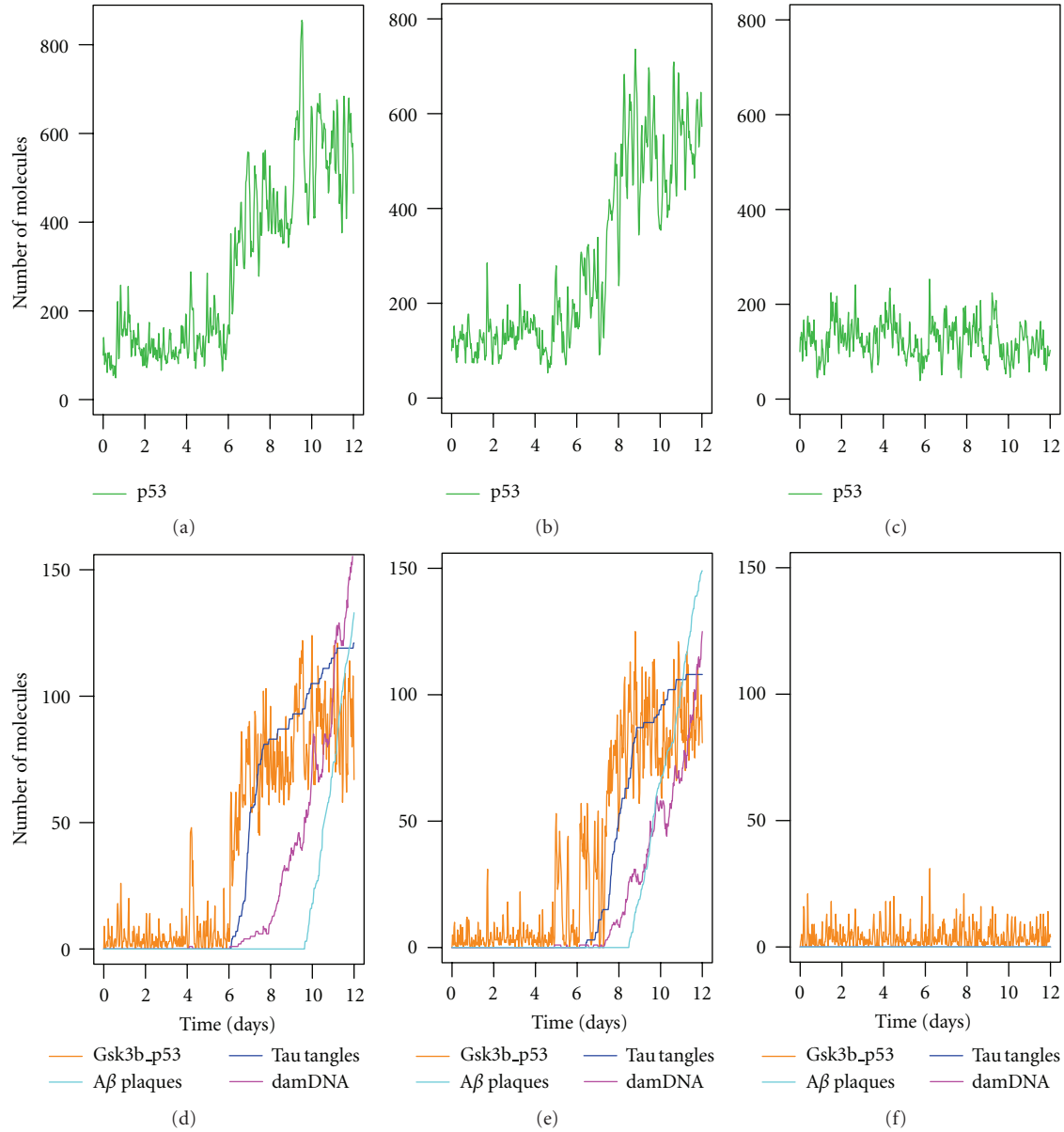
FIGURE 2: Simulation output for model with high aggregation rates and normal Aβ clearance. Three different individual simulations are shown. The plots in each column are from the same simulation. (a)–(c) Levels of p53 (total pool including bound and ubiquitinated species). (e,f) p53 bound to GSK3β (GSK3b_p53), damaged DNA (damDNA), tau tangles, and Aβ plaques are shown.

of simulated cells accumulate both plaques and tangles due to stochastic DNA damage which leads to increased levels and activation of p53 (Figures 2(a), 2(b), 2(d) and 2(e)). The model predicts that as a result of p53 activation, GSK3β activity increases resulting in increased phosphorylation of tau and formation of tau tangles. In addition, increased p53 and GSK3β activity result in increased production of Aβ which then aggregates to form plaques. Interestingly, the model predicts that tau tangles precede Aβ plaques suggesting that plaques and tangles are formed independently. The increase in Aβ also leads to more ROS and further DNA damage which in turn leads to further activation of p53 and a cycle ensues. Increasing the clearance rate of Aβ, by two orders of magnitude, at day 0 prevents any accumulation of plaques

or tangles and p53 levels remain low over a simulated 12-day period (Figure 3, green curve and Figure 4(a)). This supports the hypothesis that the increase in ROS via Aβ reinforces the cycle by activation of p53 and GSK3β as suggested above.

3.2. Effect of Increasing Aβ Clearance at Different Time Points. It is of interest to examine the effect of increasing Aβ clearance at later timepoints, since such interventions may occur after soluble Aβ or even plaques have had time to form. Studies on Aβ immunization in mice indicate that interventions are more effective if administered early, suggesting that the load of Aβ at the time of immunization is important [22]. We therefore used the model to explore the effect of increasing the clearance of Aβ at different
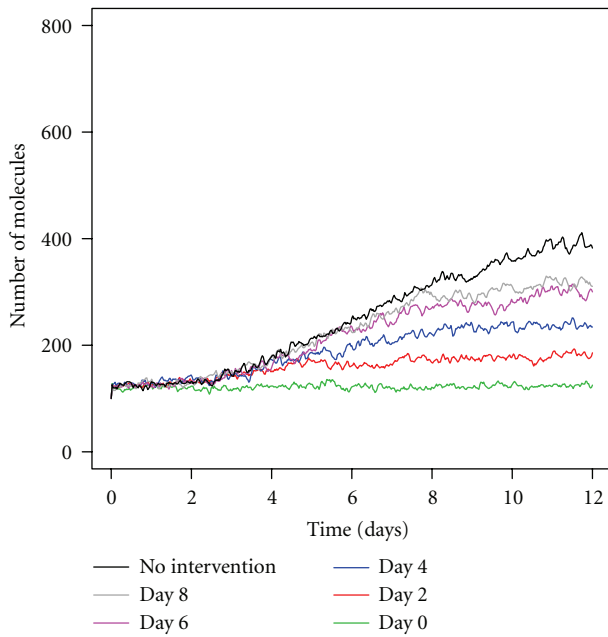
FIGURE 3: Levels of p53 under conditions of high aggregation rates and increased Aβ clearance at different time points. Each line shows the mean levels of p53 (total pool including bound and ubiquitinated species) from 100 simulations over a 12-day period.

time points (Figures 3 and 4). This was done by adding a timed event to the SBML code so that the parameter for Aβ clearance is reduced by two orders of magnitude at time 2, 4, 6, or 8 days from the start of the simulation. The model predicts that increasing Aβ clearance at early time points (up to day 4) leads to a much lower level of Aβ so that no plaques form and there are also much lower levels of tau tangles and p53 (Figures 3, 4(a)–4(c)). Note that the intervention at day 2 leads to low levels of Aβ monomers which are sufficient to slightly increase ROS levels (black curve in Figure 4(b)). Accordingly p53 levels rise slightly (red line in Figure 3) and the activity of GSK3β is increased leading to an increase in phosphorylation and aggregation of tau (Figure 4(b)). Interventions at later time points (day 6 or later) result in lower levels of plaques compared to normal Aβ clearance (compare light blue curves in Figures 4(d) and 4(e) with 4(f)) but the levels of tau tangles are not significantly lower compared to no intervention (Figures 4(d)–4(f), dark blue curves). This is due to the formation of Aβ monomers and oligomers occurring before the intervention, which leads to increases in ROS, activation of GSK3β, and increased phosphorylation of tau which is then more likely to form tangles. Figure 3 shows p53 levels start to increase after day 2 and continue to increase until the intervention of increased Aβ clearance occurs. This can be seen clearly by the fact that all curves are initially close together but as the intervention occurs, p53 levels stabilise. The model therefore suggests that even a low level of soluble Aβ monomers and oligomers is sufficient to trigger an increase in ROS, which leads to an increase in p53.

3.3. Inhibition of ROS Production via Aβ.

To confirm whether the increase in p53 is due to Aβ-mediated ROS production,

we ran 100 simulations in the model with increased Aβ clearance at day 8 and blocked the production of ROS via Aβ (by setting the parameter for Aβ-mediated ROS production to zero). Figure 5(a) shows the mean value of these simulations for p53, GSK3β bound to p53, Aβ plaques, tau tangles, and damaged DNA over a 12-day period. It can be seen that with the exception of p53, the levels of the all species shown are close to zero. So the model predicts that this intervention completely prevents the increase in DNA damage, the elevation of p53, the increase in GSK3β activity, and the formation of plaques and tangles producing results similar to increased clearance of Aβ at day 0 (see Figure 4(a)).

3.4. Inhibition of GSK3β/p53 Binding.

To examine the effect of GSK3β/p53 binding on the aggregation process we inhibited the interaction between GSK3β and p53 (by setting the parameter for GSK3β/p53 binding to zero). We ran 100 simulations with increased clearance of Aβ on day 8 (with ROS production via Aβ restored). This additional intervention also prevented the formation of plaques and tangles even though p53 levels rose during the simulation (Figure 5(b)). Therefore the model predicts that Aβ clearance at late time points may be beneficial if additional interventions are used such as simultaneously reducing ROS levels or preventing the activation of GSK3β.

3.5. Effect of Aβ Immunization on Neuronal Loss.

Cell death is not currently explicitly included in the model, but we can assume that if p53 reaches a threshold then it triggers an apoptotic pathway. Since it would be unrealistic to assign to the threshold an exact and invariable value, the threshold level of p53 is chosen from a random distribution (normal distribution, mean 600, variance 50) for each simulation run. For each simulation the level of p53 was tracked over time, starting at time zero. If the level of p53 exceeded the chosen threshold, the time at which this occurred was recorded and the simulated cell was considered to have undergone cell death at this time. The percentage of viable cells at each time point was calculated for each of the intervention times and plotted (Figure 6). The model predicts that there are no cell deaths if Aβ clearance is increased at early time points but as the intervention is increasingly delayed the percentage of cell death increases. If the intervention is as late as day 8, there is little improvement in cell viability compared to no intervention. The model therefore indicates that increased clearance of Aβ needs to occur at early time points before there is any accumulation of Aβ.

4. Discussion

The model shows that reducing the burden of Aβ reduces levels of ROS, which leads to less DNA damage, lower p53 activity, lower GSK3β activity, and reduced tau phosphorylation. If Aβ clearance is increased at early time points, there is a decrease in plaques and also a reduction in tau tangles. The model therefore does not support a dual pathway (Figure 1(b)). On the other hand, increasing Aβ clearance at late time points reduced plaque formation but did not reduce
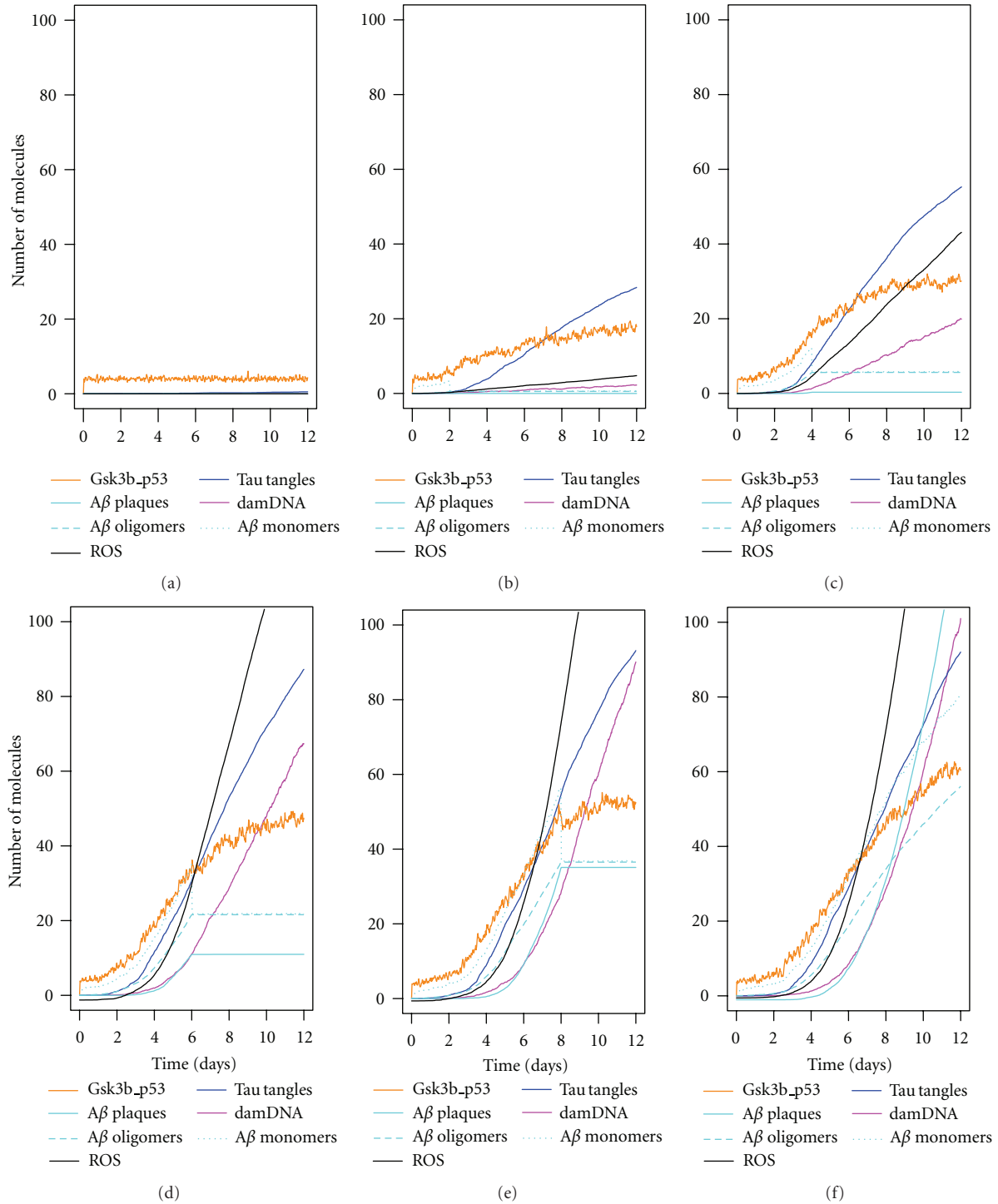
FIGURE 4: Simulation results for model with high aggregation rates and increased Aβ clearance at different time points. Each graph shows the mean of 100 simulations. The clearance rate of Aβ was increased at the following time points: (a) Day 0, (b) Day 2, (c) Day 4, (d) Day 6, (e) Day 8, (f) No intervention. p53 bound to GSK3β (GSK3b_p53), damaged DNA (damDNA), ROS, tau tangles and Aβ monomers, oligomers and plaques, are shown.

tangle formation. Neither then does the model support a linear pathway (Figure 1(a)). Rather the model supports the complex pathway where plaques and tangles can form independently due to an upstream event but with increased tangle formation in the presence of Aβ (Figure 1(c)). We

propose a new hypothesis in which the pathway between Aβ and tau is via ROS, p53, and GSK3β (Figure 7). It is important to note that GSK3β, which is shown at the top of the diagram, is not necessarily the starting point for the ensuing cascade of events. For example, the initiating event could
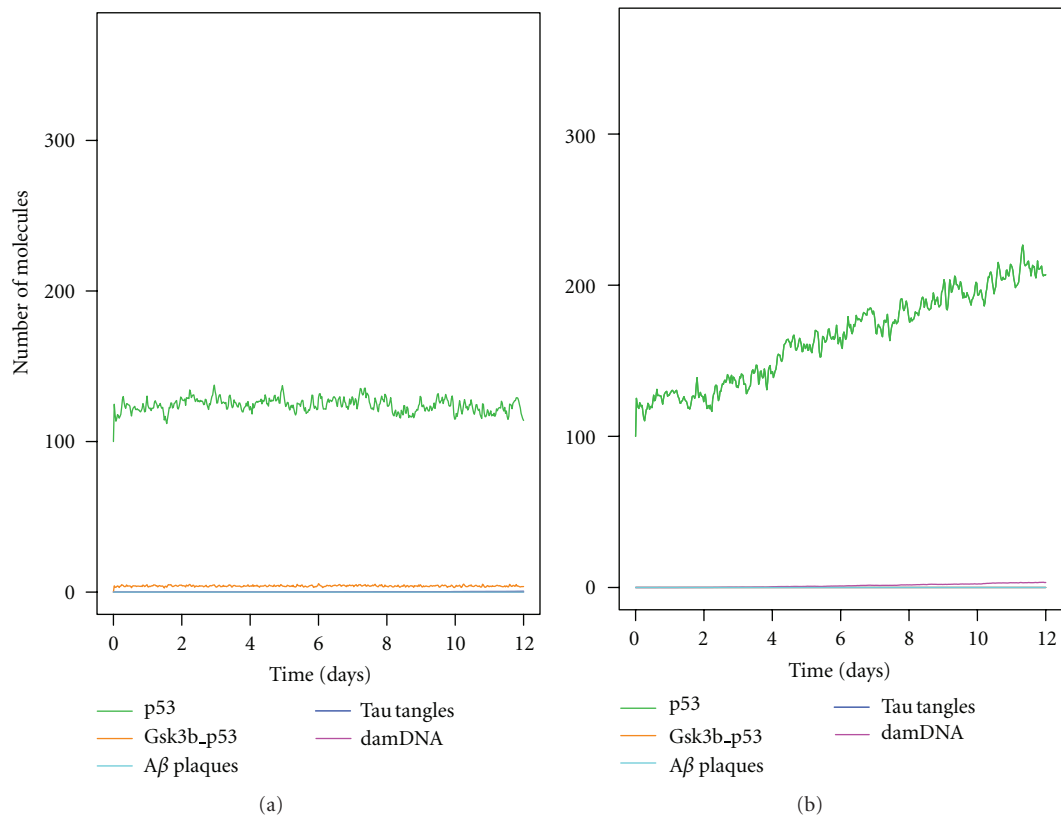
(a)

(b)

FIGURE 5: Increased Aβ clearance on day 8 with additional interventions. Each graph shows the mean of 100 simulations. (a) Blockage of ROS production via Aβ (parameter for Aβ-mediated ROS production set to zero). (b) Inhibition of GSK3β/p53 binding (parameter for GSK3β/p53 binding set to zero). Note that apart from p53, all proteins shown in the graphs have levels close to zero and so not all the lines can be seen.
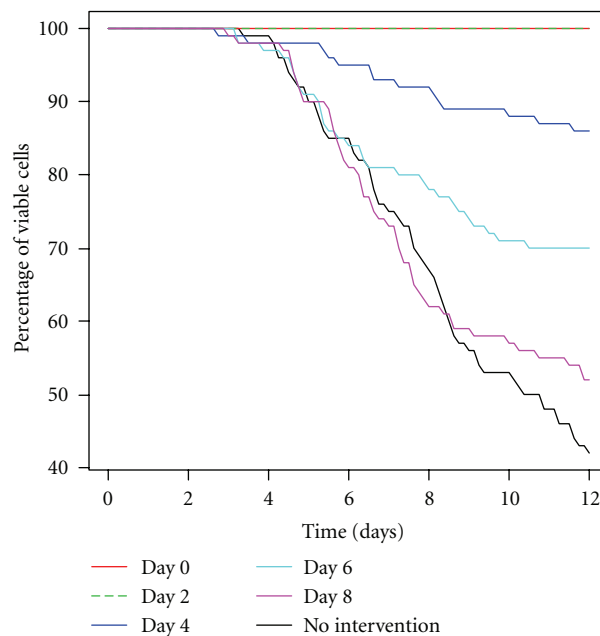

FIGURE 6: Percentage of viable simulated cells for increased Aβ clearance at different time points. Each curve shows how the percentage of viable cells (from 100 simulations) changes with time over a 12-day period when Aβ clearance is increased at days 0, 2, 4, 6, or 8 and for the normal clearance rate (no intervention).
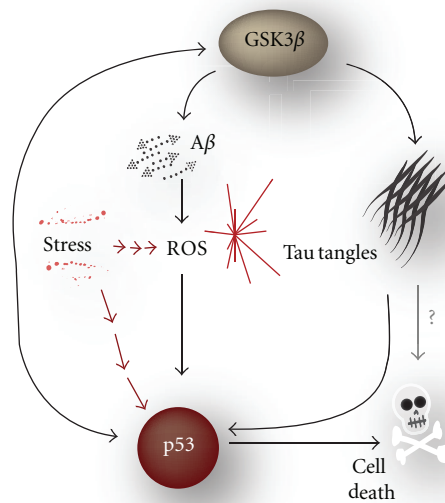
FIGURE 7: New hypotheses of AD involving GSK3 and p53. Our model supports that the pathway between Aβ and tau is via ROS, p53, and GSK3β. Note that there is a cycle in the diagram between GSK3β, Aβ, ROS, and p53 which can start at any point. Full details are in the text.

be an increase in soluble Aβ which then leads to plaques and an increase in ROS. Elevated ROS may then cause DNA damage which results in increased levels of p53, followed by increased activity of GSK3β. Finally the increased activity of GSK3β leads to tau hyperphosphorylation and tangle formation. In addition, levels of Aβ are increased and so there is a positive feedback loop which reinforces the cycle on the left. Note that GSK3β also increases p53 activity providing an additional positive feedback in the cycle. The cycle could also begin with increased ROS due to cellular stress, an increase in dysfunctional mitochondria, and/or a decline in the efficiency of the antioxidant system. Furthermore, the cycle could begin with p53 due to stress-induced DNA damage, telomere uncapping, or inhibition of the proteasome. Whatever the initiating event the positive feedback loops could promote a self-perpetuating and amplifying cascade of events that could lead to frank AD.

The model also supports the amyloid hypothesis for familial forms of the disease, since the initiating event for this form of the disease would be increased production of Aβ due to mutations in genes involved in APP processing. In this case the cycle starts with Aβ and then leads to increased ROS, DNA damage, increased levels of p53, increased GSK3β activity, and finally hyperphosphorylation of tau and formation of tangles in a seemingly linear pathway. The model also explains why tau pathology may be seen before plaques or even without plaques if the initiating event is increased activity of GSK3β, or if the cycle starts with ROS or p53. The scenario in which tangles appear without any plaques would suggest however that there must also be more efficient clearance of Aβ since an increase in GSK3β activity also increases Aβ production.

There is experimental data to support all the arrows in the diagram, however the importance of p53 in the loop has not been fully investigated. Although it is known that p53 increases the activity of GSK3β [23] and that increased p53 activity indirectly leads to tau hyperphosphorylation [24], as yet no experiments have been carried out to prove that the link between p53 and tau is GSK3β as our model suggests. This prediction could be tested experimentally by either inhibiting or overexpressing p53 in cells expressing Aβ and then measuring GSK3β activity and levels of phospho-tau.

The model is a simplification of the system but as the model is encoded in SBML, it can easily be extended to include further details. Other important components which could be added are chaperones (GSK3β is a client of Hsp90), more detail of tau regulation, the insulin pathway, and wnt signalling pathways. It would be of particular interest to include the insulin signalling pathway in order to explore the connection between AD and type 2 diabetes since GSK3β has been implicated in both diseases. Mitochondria also play an important role in the disease process. For example, damaged mitochondria may accumulate in postmitotic neurons and cause an increase in ROS which could start the vicious cycle shown in Figure 7. In addition, soluble Aβ binds to Aβ-binding alcohol dehydrogenase (ABAD) which leads to increase ROS via mitochondrial dysfunction [25, 26]. Recent data show that truncated tau and Aβ act cooperatively to impair mitochondrial function and reduce mitochondrial transport in neurons [27]. A model of mitochondrial dynamics is currently being developed and linking this with the current model will give a more complete picture of the disease process.

Aβ immunotherapy works by either active immunization with Aβ aggregates or by passive transfer of anti-Aβ antibodies. Both approaches have been shown to prevent Aβ deposition and to clear already existing plaques. Wilcock et al. showed two phases in the clearance of plaques [28]. First, there was a sharp decline in plaques 24 hours after immunization due to disaggregation and then a further decline about 3 days later due to the activation of microglia which removed the plaques by phagocytosis [28]. Our current model could be modified to mimic the immunization process by including additional reactions for plaque disaggregation and clearance. The disaggregation of plaques leads to an increase in soluble Aβ and since these may be toxic due to their interaction with mitochondria and their involvement in ROS production, our model may show that such an intervention would be less beneficial than the increased clearance of soluble Aβ. Therefore, the model could prove very useful for testing the consequences of different interventions.

5. Conclusions

Our mathematical model supports a complex pathway linking Aβ and tau via GSK3β, p53, and oxidative stress. Importantly, the pathway contains a cycle with multiple points of entry. It is this property of the pathway which enables the model to be consistent with both the amyloid hypothesis for familial AD and a more complex pathway for sporadic forms.

Acknowledgments

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