Modelling the Reactive Oxygen Species Initiated Amyloid Aggregation and Inhibitory Action of Chlorogenic Acid

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Abstract

Alzheimer’s disease (AD) is a common form of dementia which is closely linked with the reactive oxygen species (ROS) and the abnormal aggregation of amyloid beta (Aβ) protein in the human brain. Aβ protein aggregates to form plaques which deposit across the neurons and lead to neuronal cell death. Thus, Aβ protein is thought to be a major factor for AD pathogenesis. Currently, different strategies are being explored to prevent Aβ aggregation. Seeking therapeutic molecules that could inhibit the aggregation effectively, has been a major research challenge. Chlorogenic acid (CA), considered as an antioxidant, is reported to have an inhibition effect on Aβ aggregation. Herein, a previously reported kinetic model based on free radical polymerisation, assuming ROS as an initiator, is extended and used to study the Aβ aggregation and inhibitory effects of CA. Model parameter tuning is done with the experimental data to estimate the value of new parameter in the model. The simulated results from the model are observed to be in good agreement with the experimental data at a different concentration of CA. The model may also be extended to study the inhibitory effects of other drugs, such as polyphenols and metal chelators, showing a similar kinetic mechanism for inhibition of Aβ aggregation.

**Keywords**: Kinetic modelling, Aβ aggregation, drug inhibition, Chlorogenic acid.

* 1. Introduction

Alzheimer’s disease (AD) is a common form of dementia characterized by abnormal protein aggregation leading to cognitive disabilities and memory loss (Knowles et al. 2014; Mroczko et al. 2018). The late-onset disease, being more probable than early onset, affects almost 10% of the population above 65 years (Hao and Friedman 2016; Zhou et al. 2004). Currently, at least 50 million people are affected worldwide (Breijyeh and Karaman 2021).

Among the several proposed hypotheses, the amyloid beta (Aβ) cascade hypothesis has gained significant attention in previous few decades (Doig 2018; Rudge 2022). Aβ proteins are produced by a two-step sequential cleavage of amyloid precursor proteins (APP) which are sometimes cleaved by amyloidogenic pathways responsible for Aβ toxicity (Vijayan and Remya 2019). The hypothesis assumes that the excessive accumulation of Aβ leads to neuronal cell damage (Carrillo-mora and Colín-barenque 2014).

Huge *in vitro* experimental studies have been performed and corresponding mathematical models have been developed to study Aβ aggregation*.* A traditional mathematical model includes nucleation phase, elongation phase and plateau phase which are observed as a result of nucleus formation, fibril elongation and mature fibril/plaque formation respectively (Ghosh et al. 2010).

Currently, only two types of small molecule drugs, cholinergic inhibitors to cholinesterase enzyme and antagonists to N-methyl d-aspartate (NMDA), have been approved by the Food and Drug Administration (FDA, USA) to treat AD symptomatically (Miculas et al. 2023). The available therapies only temporarily relieve the symptoms and no disease modifying drug has been developed to date which can prevent the disease pathogenesis (Urbanc 2021). Based on the amyloid hypothesis, different strategies are being employed to develop anti-Alzheimer’s drugs, with mechanisms of action mainly focusing on reducing the generation of amyloid precursor protein (APP), inhibiting the cleavage of APP by inhibiting the beta and gamma secretase and preventing Aβ aggregation (Miculas et al. 2023; Wu et al. 2022).

Various mathematical models are reported to study the inhibitors’ mechanisms of action with Aβ species *in vitro* and to propose possible potential therapeutics. Therefore, a mathematical model is important to study the inhibitory action of molecule inhibitors to find the mechanism and possible therapeutics. Our previously reported kinetic model (Abdul and Garg 2023) was developed to study Aβ aggregation only. The model does not consider the effects of inhibitors and associated mechanisms of action on Aβ aggregation inhibition. The aggregation model is extended to simulate these effects and is the main scope of the current study.

The objective of the current study is to develop a model involving simple ROS initiated free radical polymerisation kinetic equations considering the effects of AD drug on amyloid beta aggregation. In this study, the proposed model deals with amyloid beta aggregation and the inhibitory action of therapeutic molecule, CA, on Aβ40 aggregation. To the best of our knowledge, this is the first ever reported model which studies the inhibition action of drug via free radical mechanism. It may give further insights to develop novel drugs for the treatment of AD.

* 1. Inhibition Model

To study the inhibitory action of the therapeutic molecule, CA, our previously reported model (Abdul and Garg 2023), originally developed for Aβ aggregation, is further extended. Mancini and co-workers (Mancini and Weaver 2018) investigated that components of coffee (e.g., CA) show inhibitory action on Aβ40 fibrillation using ThT fluorescence. Moreover, Yang and co-workers (Yang and Zheng 2018) reported that CA could inhibit Aβ40 aggregation in a dose dependent manner.

In the extended model, only inhibitory action of CA has been considered. Therefore, a kinetic reaction between CA and Aβ40 monomer has been assumed as CA reacts with Aβ40 monomer and forms a (here *M* represents Aβ40 monomer) molecule as shown below.

|  |  |
| --- | --- |
|  | (i) |

Therefore, assuming the elementary reaction mechanism, the equation for monomer concentration (modification of monomer concentration, Equation 3 by (Abdul and Garg 2023)) based on mass balance can be written as follows:

|  |  |
| --- | --- |
|  | (ii) |

where is the rate constant for monomer CA interaction.

Rate equation for CA can be written as follows:

|  |  |
| --- | --- |
|  | (iii) |

Therefore, the extended model consist of a total of 10 simultaneous differential equations, including basic model (Abdul and Garg 2023)(excluding equation for monomer in the basic model), and equations (ii) and (iii).

* 1. Results and Discussion
		1. Model tuning and validation

The experimental data (represented by diamonds in Figure 1) for CA inhibition at an initial concentration (*CA*0) of 112.9 *µ*M is used to tune the extended model. The previously reported rate parameters (*kd =5×10-8 s-1, ki = 4.5×10-8 M-1s-1,kp­= 5.8×102 M-1s-1, ktc = 4.8×101 M-1s-1, ktd ~ 0*) are used and no re-tuning is done except for the added parameter *km,* in the extended model. To solve the set of differential equations *ode23s* solver in MATLAB® is used. Model is tuned to estimate the value of the new parameter *km*. The error function (sum of the square of difference of experimental data model value data) is minimized using a nonlinear least square (LSQNONLIN) solver in MATLAB®. Experimental data shows that monomers (initial concentration of 35 *µ*M) rapidly form fibrils at t ~ 0 with concentration of 17*µ*M. Therefore, setting initial monomer concentration of 35 – 17 = 18 *µ*M, the best fit value of parameter *km* is obtained as4.810-2*M*-1*s*-1. The comparison (Figure 1) between experimental data (diamonds) and the tuned model prediction (solid line) is shown.

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 **Figure 1.** **Comparison between model output with experimental data at monomer concentration of 35** ***µ*M.** Diamonds and squares show the dose inhibition experimental data at 112.9 and 169.35 *µ*M, respectively. Solid and dashed lines represent the tuned and predicted model output at dose concentration of 112.9 and 169.35 *µ*M, respectively.

Tuned model output is observed to be in good agreement with the experimental data with only one new parameter tuning. It is noted that there is a difference between model output and the initial two experimental data points. This difference is due to only one new added parameter tuning to the model and not tuning the previously reported parameters.

* + 1. Model 1 predictions at different CA dose concentration

The experimental data is also available at another initial dose concentration (represented by squares in Figure 1). To analyze the model’s reliability, model simulations at an initial CA concentration of 169.35 *µ*M are compared with the experimental data keeping all the parameter (*kd, ki, kp, ktc, ktd, km*) values constant. After changing the initial CA concentration only, the comparison between experimental data (Figure 1, squares) and the extended model simulations (Figure 1, dashed line) are shown in Figure 1. It is observed that the model prediction for different initial concentration of CA, without any further re-tuning, agrees well with the reported experimental data.

3.3 Sensitivity of drug concentration and parameter

To check the model robustness, sensitivity analysis of initial drug concentration (CA0) and associated parameter *km*have been performed on fibril concentration. It is expected that decreasing or increasing CA0 or *km* should decrease or increase the inhibition effect of drug, respectively. Similar trends can be observed in Figure 2 (**a** is for CA0 and **b** is for *km*). It can be seen that after changing the CA0 or *km* by 20% and 180% fibril concentration gets increased or decreased, respectively, as expected.

**Figure 2. Sensitivity of drug concentration (CA0) and parameter (*km*)**

a

b

* 1. Conclusions

In this study an extended model of a basic kinetic model (which solely studies the Aβ aggregation), to study the amyloid beta (Aβ) aggregation and the inhibition effects of chlorogenic acid (CA) drugs on Aβ aggregation is proposed. The CA molecule is reported to have an inhibitory effect on Aβ aggregation. Therefore, mechanisms involving Aβ aggregation inhibition have been incorporated to modify the previously reported model. The extended model requires only one new parameter to fit the reported experimental data. The model predicts the experimental data at different monomer concentrations in good agreement. The approach provides a relatively simple and inexpensive model for studying Aβ aggregation inhibition at different conditions. The model may further be modified to study other inhibitory molecules’ effect such as, large molecules (peptide-based) and nanoparticle inhibitors on Aβ aggregation, especially when the molecule is antioxidant (works as a scavenger for ROS). Therefore, the model can be helpful in understanding the mechanism of action between Aβ and drug to develop possible therapeutics for AD in the near future.

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