Systems pharmacology of the amyloid cascade : unfolding oligomer modulation in Alzheimer's disease

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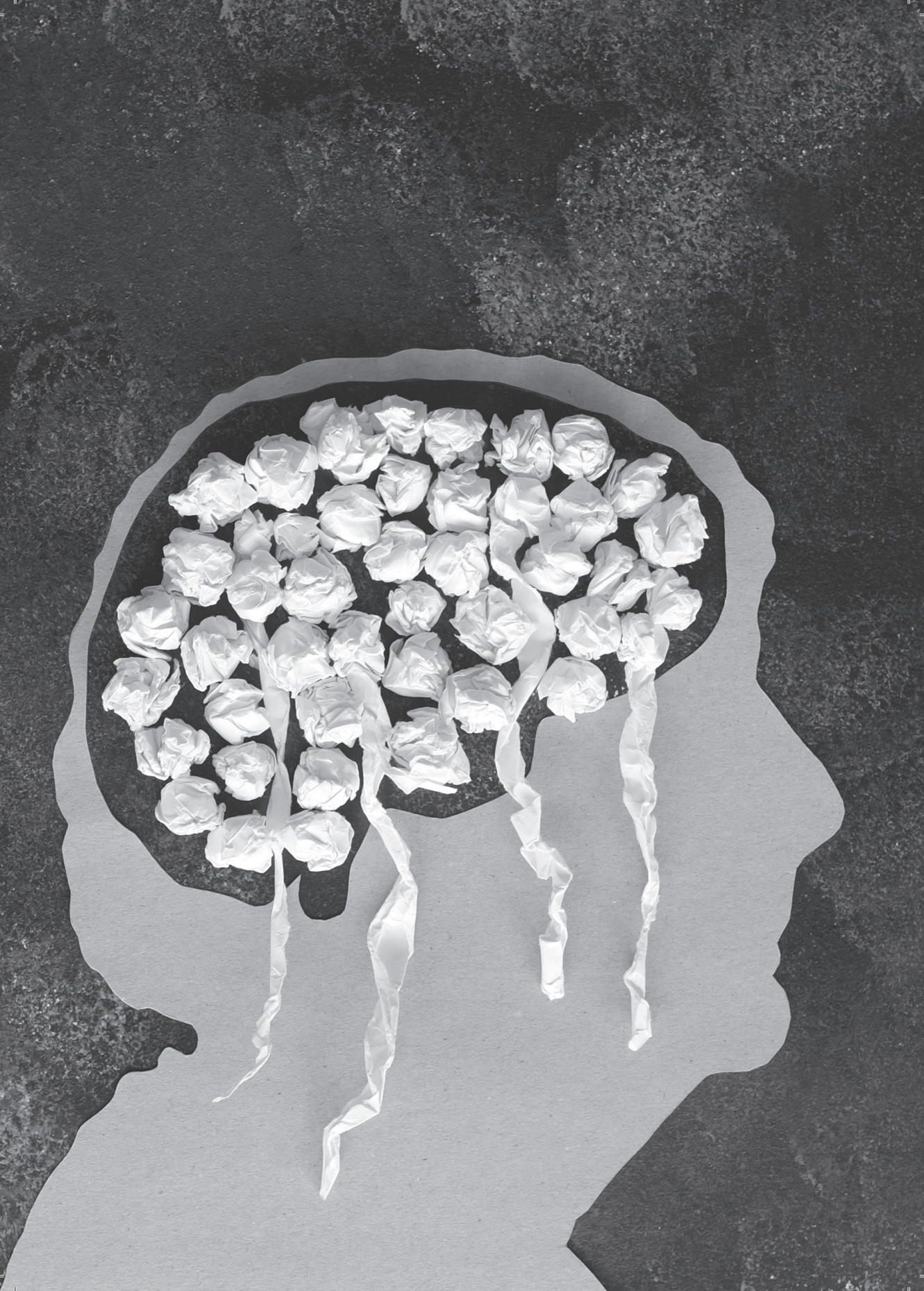
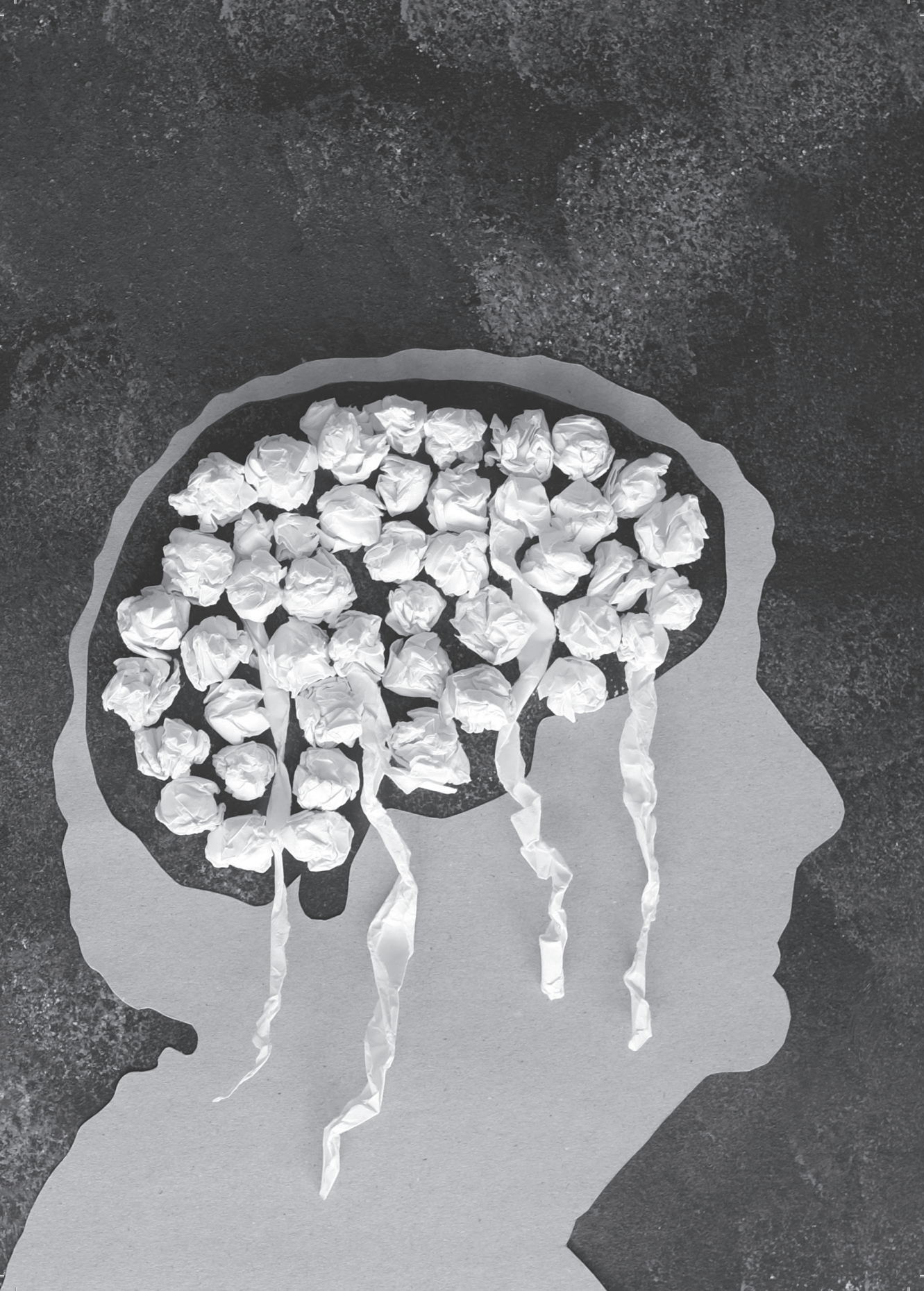


Section I

Systems pharmacology of the amyloid cascade

General introduction



Chapter 1

Systems pharmacology of the amyloid cascade

Scope and outline of investigations

Scope

The misfolding and abnormal assembly of proteins is the hallmark of various pathologies, including neurodegenerative diseases such as Alzheimer's Disease (AD)[1]. The amyloid cascade hypothesis posits that abnormal amyloid-β (Aβ) peptide processing, resulting in the deposition of Aβ in the brain parenchyma, initiates a sequence of events ultimately leading to the development of AD dementia[2]. The amyloid hypothesis provides a framework for all amyloid disorders, in which protein misfolding and different stages of aggregation are the drivers of pathological changes.

Aβ is believed to exist as a mixture of monomers, oligomers and fibrils, which are in a constant equilibrium[3]. Within this mixture, toxic soluble Aβ oligomers (Aβ_{O}) are considered to be the primary drivers of the neurodegeneration in AD brain[3,4]. One of the main therapeutic strategies for AD aims at Aβ reduction in the central nervous system (i.e. CSF and brain) through the inhibition of secretases responsible for its production[5]. Therapeutic strategies aimed at reducing the Aβ burden have the potential for eliciting a disease modifying effect, inhibiting disease progression and subsequently the prevention of the development of Aβ associated pathologies. When targeting the disease in its earliest stages, a biomarker related to the pathophysiology is needed to detect AD before symptoms as mental decline and brain damage occur. Aβ_{O} is a potential biomarker for disease progression of AD.

To date, despite decades of research, there is no treatment that halts or slows progression of the pathological cascade in AD. The development of disease-modifying therapies, such as anti-Aβ treatments, has lead to several failures. Multiple studies on the pharmacokinetics (PK) and the pharmacodynamics (PD) of Aβ production inhibitors have been reported[6,7,8,9,10,11,12]. A limitation of these studies is that they focused on the behaviour of monomeric Aβ and not at the pathologically relevant species Aβ_{O}. Moreover, these investigations focused on specific aspects of the pathology without considering the functional behaviour of the system as a whole. In this respect it is important that the amyloid cascade has the structure of a biochemical network as the rate of formation of Aβ_{O} is not determined by the activity of a single enzyme. This complicates the prediction of the behaviour of a single component, without considering the dynamic equilibrium of multiple pathways in the system, where all kinds of mechanisms contribute to the resilience of the system. Further, it may be necessary to deploy combination therapy which targets multiple parts of the biochemical network to obtain sufficient suppression of Aβ_{O}. In this respect, systems analysis is essential in order to gain insight into the expected response of

such a therapeutic intervention.

Biological and preclinical research are constantly adding new pieces of the puzzle of the amyloid cascade. The integration of pharmacological and biological information through a systems pharmacology approach has the potential to bring us closer to optimizing the therapeutic intervention to reduce $A\beta_O$ burden. A further advantage of a systems approach is that the model can be extended when new information becomes available, thereby building up and integrating the knowledge available for the β-amyloid precursor protein (APP) pathway. Such an approach will provide an adequate, mechanistic understanding of the behaviour of the APP pathway as a whole and its resilience, as opposed to the behaviour of its individual attributes, which is imperative to improve the prediction of therapeutic effects on $A\beta$ and their reflection on $A\beta_O$ levels.

The aim of the proposed investigation is the development of a systems pharmacology model describing the functioning of the APP processing pathway, with emphasis on the dynamics of the $A\beta_O$ during pharmacotherapy aimed at reduction of $A\beta$ monomers. Therefore, the objectives of the proposed investigation are:

[1] To establish a systems pharmacology model to describe in a strictly quantitative manner the biochemical network of APP processing.

[2] To predict and evaluate the effect of $A\beta$ production inhibitors, acting at different sequence in the APP processing pathway, on $A\beta_O$ concentrations.

[3] To explore other therapeutic strategies which may aid the reduction of $A\beta_O$ burden.

Outline

In **section I** the amyloid cascade hypothesis for Alzheimer's disease is presented as a theoretical framework for the modeling of drug effects in protein misfolding neurodegenerative diseases. Moreover, the use of Aβ as biomarker for disease progression and as the scientific basis of therapy leading to a decrease in Aβ production is considered. In addition, the use of systems pharmacology modeling to provide a quantitative understanding of the modulation of the amyloid cascade is discussed (**Chapter 2**).

In **section II** a systems pharmacology model is presented describing the changes of the β-amyloid precursor protein (APP) pathway in response to relevant β- and γ-secretase inhibitors, in cisterna magna ported rhesus monkeys[13]. In (**Chapter 3**) a systems pharmacology model is presented that describes the changes in APP metabolites to β-secretase (BACE1) inhibition[14]. Based on monomeric APP metabolite data an Aβ oligomer pool was identified through modeling, suggesting that Aβ production inhibition may have the ability to reduce Aβ oligomeric forms as well.

Next, the systems pharmacology model was extended to account for tracer dynamics in response to BACE1 inhibition throughout the APP pathway (**Chapter 4**). In the past, a stable isotope labeling kinetic (SILK) platform to measure Aβ was developed by Bateman and colleagues[15]. As both SILK data and absolute concentration measurements of APP metabolites from an enzyme linked immunosorbant assay (ELISA) were available from the same study, and both were measurements of the same biological system, it was of interest to compare these. The systems model was able to integrate the two types of data and describe seven biomarkers successfully, which facilitated a comparison of absolute concentrations of APP metabolites with the tracer kinetic data. In addition, the combined analysis confirmed the biological system that was identified based on absolute concentrations of APP metabolites only.

Concurrently, the systems pharmacology model was extended to describe the Aβ responses to include γ-secretase (GS) inhibition (**Chapter 5**)[16]. This extension is considered to be an essential addition as it enabled the separation of BACE1 and GS sequential cleavage steps. Furthermore, differences in Aβ response and anticipated effect on Aβ oligomers (Aβ_{O}) between BACE1 and GS inhibition were assessed. The predicted effect on Aβ_{O} was explored which was quantified at a later stage *(vide infra)*.

In **section III** the developed systems pharmacology model was applied to Aβ oligomer data. In a cross-over study, changes in Aβ oligomer levels in CSF were quantified in response to BACE1 and GS inhibition. This enabled the verification of the systems

model predictions of Aβ_{O} response to BACE1 (**Chapter 6**) and GS inhibition (**Chapter 7**). Using this data, it could be determined that BACE1 inhibition is equivalent to GS inhibition with regards to oligomer response. In addition, in this study, for the first time APP metabolite responses to GS inhibition upstream of the GS cleavage step were measured. This facilitated the identification of a homeostatic feedback mechanism in the APP pathway (**Chapter 7**).

In **section IV** the summary and general discussion are provided. In **Chapter 8** the results of the presented research are summarized. The systems pharmacology model was used to simulate the effects of Aβ elimination enhancers on Aβ_{O}, as well as combination therapy. In addition, future perspectives are addressed. The final goal would be to utilize the systems pharmacology model in translational pharmacology to anticipate Aβ response of new drug candidates in human. Hence, the scaling-up of the systems pharmacology model developed in rhesus monkeys for application in humans is discussed. Additionally, a number of potential applications of the model in the design and development of therapeutic interventions for AD are considered.

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