The Role of Circulating Aβ Seeds in the Progression of Cerebral Amyloidosis

Nazaret Gamez1,2 and Rodrigo Morales1,3

1Department of Neurology, The University of Texas Health Science Center at Houston, Houston, TX, USA. 2Dpto. Biología Celular, Genética y Fisiología, Instituto de Investigación Biomédica de Málaga-IBIMA, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain. 3Centro Integrativo de Biología y Química Aplicada (CIBQA), Universidad Bernardo O’Higgins, Santiago, Chile.

ABSTRACT: While understudied, it is suspected that peripheral Aβ peptides affect Alzheimer’s disease (AD)-associated pathological changes in the brain. The peripheral sink hypothesis postulates that the central and peripheral pools of Aβ co-exist in equilibrium. As such, cerebral amyloid levels may be modulated by intervening circulating Aβ. In this commentary, we discuss relevant literature supporting the potential role of peripheral Aβ in exacerbating brain amyloidosis in both humans and mouse models of AD. Moreover, we highlight the need to further understand the mechanisms by which circulating Aβ peptides may reach the brain and contribute to neuropathology. Finally, we discuss the implications of targeting peripheral Aβ as a therapeutic approach in treating AD.

KEYWORDS: Alzheimer’s disease, neurodegeneration, prion, amyloid-beta, peripheral tissues

Background
Alzheimer’s disease (AD) is a proteinopathy characterized by the abnormal accumulation of misfolded proteins, such as amyloid-beta (Aβ) and tau, in the brain. While the etiology of AD is still unknown, Aβ aggregates are considered the triggers of a cascade of events leading to tau pathology, neuroinflammation, synaptic loss, neurodegeneration, and cognitive decline. The progression of Aβ and tau pathology throughout the brain may be explained by their prion-like properties. As such, it is thought that the spreading of brain pathology in AD is mediated by the self-propagation of misfolded protein aggregates, or “seeds,” that template the further misfolding and aggregation of natively folded proteins across brain regions. The protein “seeding” concept has gained special interest due to findings showing that cerebral amyloidosis can be exogenously exacerbated after the intracerebral administration of Aβ seeds into transgenic (Tg) mouse models of AD.1,2 Moreover, additional studies have experimentally demonstrated that brain amyloidosis can be induced through seeding in mice that normally do not do it.5 This “seeding” mechanism resembles that observed with infectious prions.5 Cumulative findings suggest that disease mechanisms associated with protein misfolding can be initiated and/or accelerated by the exogenous administration of similar proteinaceous particles.

Role of peripheral Aβ seeds in the progression of brain amyloidosis

Intriguingly, Aβ aggregates have been found not only in the brain of AD patients but also throughout the periphery, including vascular tissue, skeletal muscle, pancreas, and liver.6 Whether peripheral Aβ seeds play a role in exacerbating cerebral amyloidosis via seeding remains contentious. In this context, our recent study7 demonstrated that intraperitoneal, intramuscular, and ocular (eye drops) administration of Aβ-seed laden brain extracts accelerated brain amyloidosis in a mouse model of AD. Not only do our results confirm the efficacy of intraperitoneal seeding, as observed by Eisele et al.,8 but also bring to light two other relevant peripheral routes previously shown to be involved in prion infections.9,10 These results strongly suggest that Aβ aggregates present in peripheral tissues may have an effect on the progression of disease-associated pathological changes. Moreover, Eisele et al. were able to detect intraperitoneally inoculated Aβ in peripheral tissues, including the liver and spleen, and, more interestingly, in blood monocytes up to 1-week post-administration, suggesting a potential mechanism of Aβ seed transportation from the periphery to the brain via circulating macrophages.8 In support of these findings, in our study,7 we observed a significant increase in seeded Aβ deposits associated with cerebral blood and meningeal vessels in all the experimental animals that underwent peripheral induction of brain amyloidosis. Interestingly, blood cells have been implicated as a major avenue of peripheral Aβ production.11 In a recent study, bone marrow cell transplantation from APPsw/PS1dE9 mice into wild-type (WT) mice resulted in continuous production of human Aβ in the brain and caused AD-associated neuropathology in WT recipients.12 Likewise, Lam et al.,12 who developed a Tg mouse model in which the expression of human...
APP is absent in the brain and largely limited to the liver, found that aged mice displayed human Aβ deposition in the brain, suggesting that liver-derived Aβ peptides can enter the CNS and facilitate pathological changes. These data highlight the possible role of the vascular system in not only generating but also transporting peripheral Aβ aggregates to the central nervous system (CNS), where protein seeding mechanisms likely result in parenchymal amyloidosis (Figure 1A).

The peripheral sink hypothesis postulates that central and peripheral levels of Aβ are in equilibrium. As such, it is believed that an increase/reduction of peripheral Aβ may result in higher/lower levels of Aβ in the brain. In this context, numerous studies have evaluated the role of circulating Aβ on brain amyloidosis. One report by Bu et al. supports this notion by using a parabiosis model linking APPswe/PS1dE9 Tg and WT littermates. They observed that parabiotic WT animals developed parenchymal and vascular Aβ deposition as well as other AD-associated pathologies. Interestingly, circulating fluor-labeled Aβ was detected in the brain of parabiotic mice and thought to be the trigger of AD pathogenesis. Blood Aβ levels have been extensively examined in the Tg2576 animal model. To further elucidate whether circulating Aβ seeds present in the blood of old Tg2576 are able to promote amyloid pathology progression in non-disease-affected animals, we performed blood infusion experiments in young Tg mice. Our results demonstrated that the recipient animals displayed a significant acceleration of cerebral amyloidosis after receiving 2 transfusions of old-Tg blood. Unexpectedly, intravenously infused mice did not display vascular amyloid deposits in the brain. This might be explained by the fact that circulating Aβ were suspected to be composed mostly by oligomeric species, which may penetrate the CNS of recipient animals, diffuse throughout the brain parenchyma, and then seed amyloid pathology. Nevertheless, the isolation of misfolded Aβ in the blood of animal models and humans remains a challenge and several groups are actively researching to develop methods able to identify these disease-relevant particles in this bodily fluid.

Although the aforementioned studies support the idea that circulating Aβ modifies AD pathology in the brain, there are some reports that have not been able to recapitulate these findings. For instance, Fukuchi et al. found that APP-C99 Tg mice do not develop cerebral amyloidosis despite presenting high levels of circulating Aβ. In a more recent publication, Brackhan et al. generated 13C-lysine-labeled human Aβ from Tg mouse brain extracts that were intraperitoneally injected into young Tg mice. They detected the inoculated Aβ in the liver and lymphoid tissues but not in the brain of treated animals. They concluded that intraperitoneally administrated Aβ does not reach the brain from the periphery nor does it play any significant role in exacerbating brain amyloidosis. Conversely, studies in guinea pigs and nonhuman primate models of cerebral amyloidosis have found that peripherally administered...
labeled-Aβ reaches the brain, contributes to parenchymal amyloidosis, and co-localizes with Aβ plaques20,21 (Figure 1A). The discrepancies noted by the study from Brackhan et al. may be explained by the source of the inoculum and/or the utilization of specific Tg mouse models that might generate distinct Aβ species that therefore result in differences in the rate of amyloid pathology progression.

Furthermore, our study utilized Aβ-containing brain homogenate (BH) as inoculum. Considering this, it is plausible that other components of the BH (including lipopolysaccharides, inflammatory cytokines, cell-toxic products, etc.22) may influence cerebral amyloidosis in a manner distinct from Aβ seeding. While the effect of these other factors remains under active investigation, studies with purified recombinant Aβ have demonstrated a similar exacerbation in brain amyloidosis when intravenously inoculated into Tg mice (Morales et al.17 and unpublished data).

Interestingly, the experimental animals described in our peripheral administration study7 displayed a variety of Aβ-neuropathological profiles and brain tropisms depending on the peripheral route of inoculation. While in prion diseases different tropisms have often been found to correlate to different prion strains, at present we lack the evidence to claim that specific peripheral routes favor different strains of Aβ that may exist in the original inoculum. Alternatively, the distinct tropisms observed may be due to varying efficacies in reaching the CNS depending on the route of peripheral inoculation.

Importantly, our study7 considered oral administration as an additional peripheral pathway. We performed oral gavages of highly concentrated Aβ-containing BH from mouse and human origin into experimental mice. Although different dosages were tested, orally inoculated animals did not present significant neuropathological changes to that observed in non-injected control mice.7 We hypothesize that orally administered Aβ seeds may be susceptible to gastrointestinal degradation and/or unable to cross the epithelial layers of the gastrointestinal tract. Based on prion studies, prion inoculation into the tongue of hamsters is 100,000-fold more efficient than oral ingestion.23 Current experiments in our laboratory are ongoing to study whether intra-tongue inoculation of Tg BH results in an effective route to transmitting AD phenotypes into Tg2576 animals. This may provide additional information on the capability of misfolded Aβ particles to spread across different tissues.

Together, these findings shed light on the complexity of AD pathogenesis and the role that circulating Aβ may play in neuropathology and disease progression. Further investigation is needed to decipher how systemic Aβ reaches the CNS and the potential mechanisms underlying peripherally seeded brain amyloidosis.

**Potential avenues for AD therapeutic interventions**

Reservoirs of Aβ can be found in several non-neuronal peripheral tissues that likely contribute to an active interchange of these peptides between the brain and the periphery.6 It has been hypothesized that Aβ levels in the CNS and the periphery maintain an equilibrium state.14 This equilibrium is dependent, among other factors, on the capacity of Aβ to cross the blood-brain barrier (BBB) from the brain to the circulatory system and vice versa. Moreover, the efflux of brain-derived Aβ may be mediated by the blood-CSF barrier, the arachnoid villi, and the lymphatic pathway.14 It is thought that an imbalance of Aβ production and clearance may drive abnormal accumulation of Aβ in the brain and AD pathology progression.

Considering what was discussed above, targeting peripheral Aβ has emerged as an unconventional yet promising approach in the treatment of AD. The therapeutic implication of the peripheral sink hypothesis suggests that peripheral antibodies can be utilized to sequester circulating Aβ thus lowering its concentration in the blood.24,25 This shift in the dynamic equilibrium between the Aβ concentrations in the brain and periphery may cause a net efflux of Aβ from the brain which may reduce plaque load and ameliorate pathology. As such, the reduction of Aβ peptides in the periphery may be a viable mechanism for reducing Aβ related pathology in the brain.24,25 Further support for this notion comes from the observation that patients who have undergone hemodialysis, effectively clearing peripheral Aβ, exhibit decreased Aβ deposition in the brain.26 Congruently, multiple studies have demonstrated that impairment of peripheral Aβ clearance exacerbates AD pathology in the brain.27-29 (Figure 1B). These studies suggest that the modulation of peripheral or circulating Aβ merits further investigation as a promising therapeutic avenue to reduce amyloid burden in the brain.

**Concluding Remarks**

Our original publication entitled “Transmission of cerebral amyloid pathology by peripheral administration of misfolded Aβ aggregates,”7 as well as many others,8,11-13,15,17 provide evidence that peripheral Aβ seeds play an important role in the progression of cerebral Aβ amyloidosis. This is of special interest to further study AD pathogenesis, as not only brain-derived but also circulating Aβ peptides may drive both central and systemic AD-associated features. Moreover, targeting Aβ from the periphery might open novel avenues for promising diagnostic and therapeutic interventions.

**Author Contributions**

NG wrote the manuscript and prepared Figure 1. RM reviewed and edited the manuscript, and approved its final version.

**ORCID id**

Rodrigo Morales [https://orcid.org/0000-0001-7766-5770](https://orcid.org/0000-0001-7766-5770)

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