Extracting β-amyloid from Alzheimer's disease

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The amyloid hypothesis of Alzheimer's disease (AD) posits that extracellular plaques comprised of the β-amyloid (Aβ) peptide are a root cause of neuronal loss in AD (1). To date, three therapeutic strategies targeting Aβ have been used: (*i*) inhibiting the production of Aβ, (*ii*) inhibiting the oligomerization of Aβ, and (*iii*) promoting the clearance and/or degradation of Aβ. None have led to a therapeutic or preventive medication for AD. As an alternative approach, natural compounds have demonstrated remarkable promise in diseases ranging from cancer to diabetes. In PNAS, Sehgal et al. (2) describe how an extract from the root of *Withania somnifera* (WS; also known as Ashwagandha or Indian ginseng) reverses AD pathology via the peripheral clearance of Aβ.

Building on previous work by Kuboyama et al., who demonstrated that WS extracts promote neurite outgrowth under Aβ-induced neurodegeneration (3, 4), Sehgal et al. ask whether these same compounds could reverse the behavioral and pathological characteristics of AD (2). Treatment of AD mouse models with WS reversed deficits in spatial learning and memory and reduced Aβ plaque load in the brain. Rather than affecting the generation of Aβ, WS probably promoted the disassembly of toxic Aβ oligomers. Suspecting a possible efflux from the brain, the authors found that treatment with WS induced the expression of low-density lipoprotein receptor-related protein 1 (LRP1) (5), which carries neuronal Aβ into the periphery. The effects of WS extract were mediated by hepatic LRP1 and soluble LRP1 in the plasma, rather than by brain LRP1, highlighting the potentially dramatic effect of peripheral clearance of Aβ even in the absence of changes in clearance mechanisms in the brain. Taken one step further, this may offer great therapeutic promise for AD, as increasing the effective clearance of Aβ by the liver would reduce the need to develop therapeutic compounds that can cross the blood–brain barrier (BBB).

Aβ is generated through the sequential proteolysis of the amyloid precursor protein by two proteases: β- and γ-secretases. Alternatively, amyloid precursor protein can first be cleaved by α-secretase, effectively preempting its cleavage by β-secretase. Secondary cleavage by γ-secretase can yield multiple Aβ species of varying C termini, the two most common being Aβ40 and Aβ42, with the Aβ42/40 ratio strongly correlating with AD pathology (6). One therapeutic strategy would be to target the secretases responsible for Aβ generation. However, the crosstalk between multiple isoforms and multiple substrates and the shared binding pockets of β- and γ-secretases with other aspartyl proteases present a major hurdle, such that pharmacological manipulation of these proteases has off-target effects on other essential pathways (7). For example, γ-secretase has myriad substrates, one of the most important being Notch, which is critical in cellular development and differentiation. Indeed, γ-secretase inhibitors frequently display gastrointestinal, immunological, and cutaneous side effects, suggesting that the blanket inhibition of γ-secretase may be a therapeutic dead end. This has led to the development of Notch-sparing γ-secretase inhibitors and γ-secretase modulators, the latter of which alter the γ-secretase cleavage site so as to limit the production of Aβ42. γ-Secretase modulators and Notch-sparing γ-secretase inhibitors have shown conceptual promise but have yet to prove their clinical usefulness. One alternative strategy would be to increase the activity of α-secretase, which belongs to the family of a disintegrase and metalloprotease proteins. These proteases, however, also have a broad and overlapping target spectrum, making rational drug design with high specificity problematic at best. Finally, development of secretase inhibitors must be able to clear the BBB, thus posing a limitation in drug design. Indeed, strategies that target the secretases have yet to yield a potent, marketable AD drug despite more than 10 years of development.

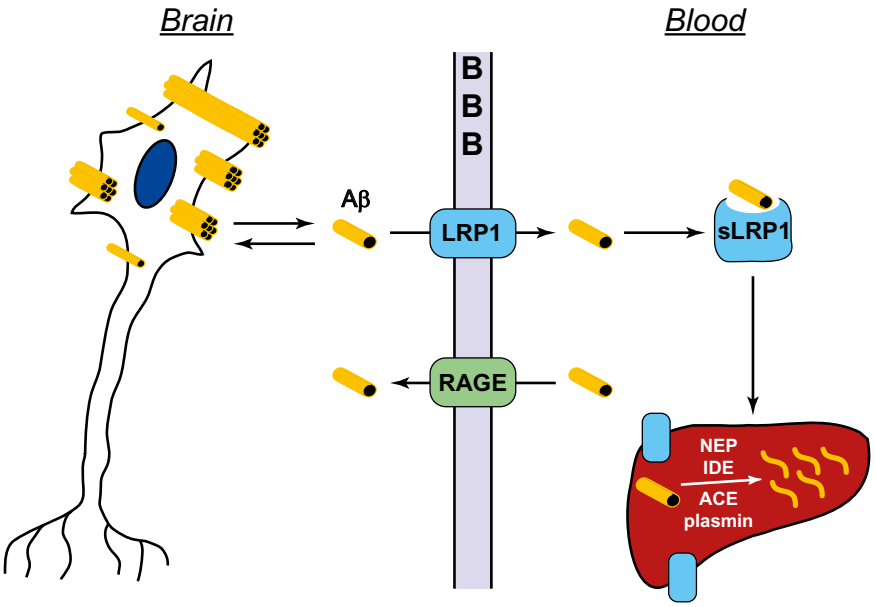

Fig. 1. Peripheral clearance of Aβ. In the brain, Aβ can oligomerize to form higher-order structures, including protofibrils and fibrils. LRP1 shuttles monomeric Aβ across the BBB and into the blood; RAGE is responsible for Aβ influx. sLRP1 and hepatic LRP1 assist in Aβ degradation via several Aβ-degrading enzymes, including NEP, insulin degrading enzyme (IDE), angiotensin converting enzyme (ACE), and plasmin. Sehgal et al. (2) find that WS extract reverses AD pathology by up-regulating sLRP1 and hepatic LRP1, thereby shifting the equilibrium to reduce the total Aβ load and oligomerization.

Another alternative strategy would be to target the oligomerization of Aβ. Aβ peptides, in particular Aβ42, are prone to aggregation. Aβ monomers aggregate to form Aβ oligomers, protofibrils, and fibrils in vitro, with Aβ oligomers deemed the most neurotoxic species (8). A number of peptide-based and non–peptide-based inhibitors of fibrillogenesis have been developed, although few have made it to clinical trial (9). Immunotherapy targeting

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Aβ has also been attempted, although the risks of meningoencephalitis and cerebral hemorrhage have given pause to this strategy (10, 11). The ineffectiveness of passive immunization may be a result of drug trials having started after the damage has already been done, arguing for prophylactic treatment instead (12). Thus, directly targeting excessive Aβ accumulation after it has occurred has proven to be problematic.

One final therapeutic strategy would be the clearance and degradation of Aβ, and it is in this respect that the work by Sehgal et al. holds promise (2). Two types of proteins mediate the transport of Aβ across the BBB: (*i*) LDL receptor-related proteins, i.e., LRP1 and VLDLR, which transport Aβ into the blood causing efflux; and (*ii*) receptor for advanced glycation end products (RAGE), which mediates Aβ influx (Fig. 1) (13). In AD, LRP1 expression is decreased and RAGE increased, highlighting the importance of the clearance arm of the Aβ hypothesis (14). Membrane-bound LRP1 is found in the brain capillary endothelium, where it mediates transport of Aβ across the BBB and into the blood. LRP1 can also be cleaved to form a soluble product, soluble LRP1 (sLRP1), which may act as a peripheral sink to sequester Aβ (13). Hepatic LRP can then assist in the degradation of Aβ in the liver by proteases, including neprilysin (NEP) (15). Targeting the clearance of Aβ, then, rather than targeting the secretases or Aβ itself, bypasses the complications of off-target effects and developing compounds that cross the BBB, hence the interest in the study of Sehgal et al. (2).

In their work (2), Sehgal et al. find that WS extract facilitates the efflux of Aβ from the brain to the blood, suggesting clearance as the responsible mechanism. Intriguingly, LRP1 expression increases in endothelial cells but not in neurons, and liver LRP1, plasma sLRP1, and liver NEP are similarly increased early in treatment. WS also promotes the disaggregation of oligomers in the cortex, presumably by shifting the equilibrium to monomeric Aβ, which is readily cleared by LRP1. Finally, knockdown of LRP1 in the liver, but not in the brain, abrogates the effects of WS, whereas inhibition of NEP has no effect, thus implicating hepatic LRP1 as the main effector through which WS facilitates the clearance of Aβ. Perhaps most importantly, the identification of drug actions outside the brain may offer particular therapeutic promise, as obviating the creation of compounds that cross the BBB opens the chemical space for AD drug design.

Sehgal et al. find that WS extract facilitates the efflux of Aβ from the brain to the blood.

Plant extracts offer a unique alternative therapeutic strategy to otherwise intractable diseases. However, whether WS offers a true therapeutic avenue for the treatment of AD has not been demonstrated. Although Ashwagandha is apparently well tolerated, the dosages used in the current study are quite high, as the authors admit (2). It is possible, however, that a trace compound or metabolite from the extracts, rather than the main components (withanolides and withanosides), mediates the effects of WS. Isolating the active constituent(s) of WS extract would also help prevent possible off-target effects. Although WS bypasses the secretase pathways, LRP1 has many other ligands, including apolipoprotein E, α2-macroglobulin, and tissue plasminogen activator (16). This still poses a potential problem for off-target effects. Finally, the mouse models used in this study are genetically compounded to provide robust Aβ production and aggregation (17). Thus, the therapeutic effect on more physiologically relevant models—and ultimately in human clinical trials monitoring the disappearance of Pittsburgh compound B-positive amyloid deposits—would substantiate the use of WS for AD.

In any case, the work proposed by Sehgal et al. (2) provides several promising strategies for AD therapy. First, this work suggests that it may be worthwhile to revisit strategies that take advantage of a peripheral Aβ sink, (e.g., by sLRP1 and the hepatic clearance of Aβ). A similar concept has been proposed for anti-Aβ immunotherapies, which aim to absorb Aβ monomers and oligomers to prevent their accumulation in the brain (18). Second, preventive measures in the presymptomatic stages of AD may offer more therapeutic promise than treatment in symptomatic stages (19). In this regard, natural compounds for which innocuous long-term use in human populations has already been documented might be more tolerable and acceptable in disease prevention. A daily, or even only periodic, preventative regimen of the active compound of WS extract could be less invasive, more cost-effective, and less prone to undesirable side effects than repetitive immunotherapy. Thus, the work of Sehgal et al. (2) provides a compelling argument to look to the natural world—be it plants, marine organisms, or microorganisms—for alternative therapeutic agents for AD.

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