

## CLINICAL IMPLICATIONS OF BASIC RESEARCH

## Attacking Amyloid

Jeffery W. Kelly, Ph.D.

Protein aggregation seems to cause numerous neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and familial amyloid polyneuropathy and cardiomyopathy. Once initiated, the aggregation of individual proteins into amyloid fibrils is difficult to inhibit with small molecules (Fig. 1), owing to the large protein surfaces involved, the plasticity of amyloid structures, and the low binding affinities of known inhibitors. A recent study, however, gives cause for hope. Gestwicki and colleagues<sup>1</sup> report that small, bifunctional organic molecules may sterically block the growth and adverse effects of fibrils.

Alzheimer's disease appears to be caused by the misassembly of the  $\beta$ -amyloid peptide ( $A\beta$ ) into an amyloid structure that is rich in  $\beta$ -sheets. This thermodynamically favorable process can occur in the presence of typical inhibitors designed to cap the growing end of the fibril, because such inhibitors tend to dissociate, owing to their high dissociation constants (1 to 10  $\mu$ M; a high dissociation constant correlates with weak binding). Moreover, it is likely that amyloidogenesis can proceed even if the inhibitors are bound to amyloid fibrils, thanks to the large surfaces involved in relation to the small size of the inhibitors and to the plasticity of the amyloid structure.

Despite substantial effort, it has proven very difficult to develop agents that inhibit the aggregation of amyloidogenic peptides that are natively unfolded and thus do not assume a well-defined native structure, such as  $A\beta$ .<sup>2</sup> In contrast, high-affinity inhibitors of amyloidogenesis have been developed for amyloidogenic proteins whose native state is structurally well defined. These inhibitors, called "native-state kinetic stabilizers," stabilize the normal fold by raising the kinetic barrier associated with the formation of amyloid and are being evaluated in clinical trials of familial amyloid polyneuropathy and cardiomyopathy.<sup>3</sup>

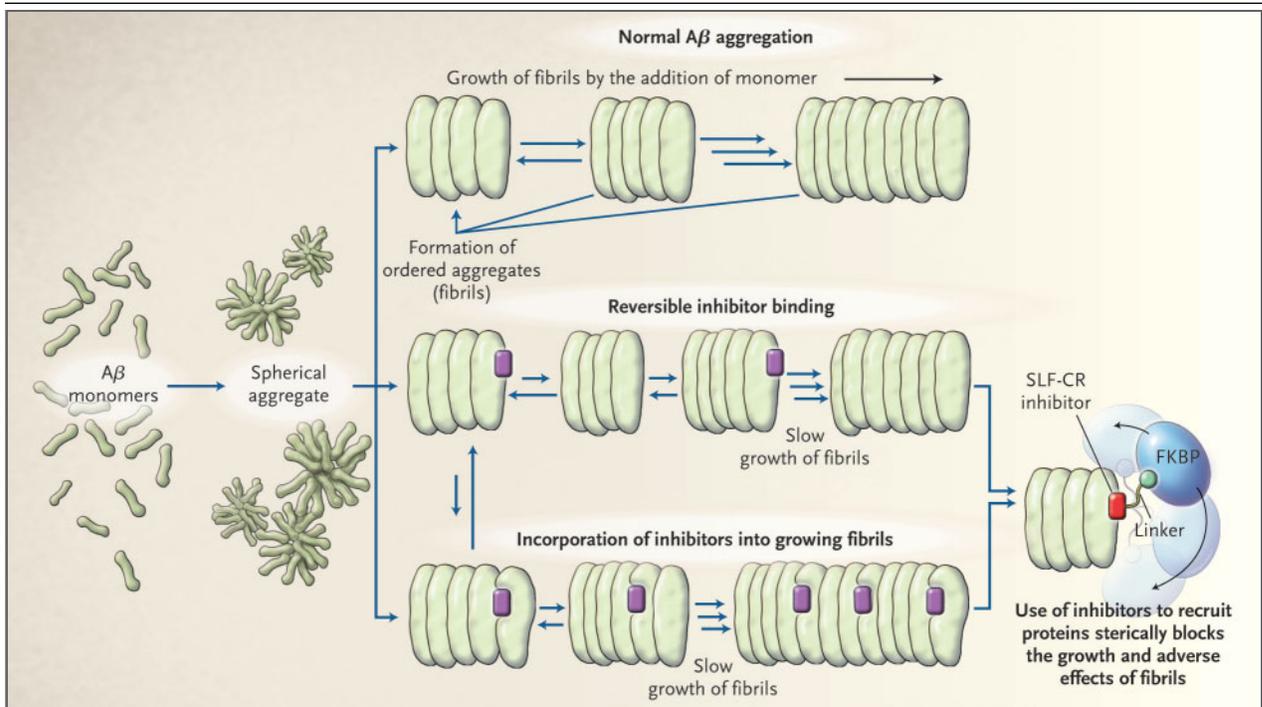
Since  $A\beta$  does not have a well-defined structure in the cerebrospinal fluid, investigators have resorted to searching for small molecules that block early steps in the multistage process of  $A\beta$  amyloidogenesis. Dyes such as Congo red used by pathologists to visualize amyloid are moderate inhibitors

of  $A\beta$  amyloidogenesis and are used as positive controls in screening. Most of the  $A\beta$ -aggregation inhibitors identified so far are flat, aromatic molecules with activity in the micromolar concentration range. In the most rigorous assays, however, none of these agents seem to prevent  $A\beta$  amyloidogenesis *in vitro*. They merely slow the process, unlike kinetic native-state stabilizers, which completely or nearly completely prevent amyloidogenesis at similar concentrations.<sup>3</sup>

What we need is a potent, orally available inhibitor of  $A\beta$  amyloidogenesis that is small enough to cross the blood-brain barrier but big enough to interfere with  $A\beta$  amyloidogenesis. Enlarging the currently available inhibitors to block amyloidogenesis sterically would probably compromise their oral bioavailability and permeability. Gestwicki and colleagues offer a simple solution to this apparent conundrum. They chemically modified the structure of Congo red to include a remote motif that can bind to and recruit the forkhead binding protein (FKBP), a large, soluble endogenous protein not involved in the formation of amyloid. The Congo red substructure serves to cap the elongating amyloid fibril, while the remote motif binds with high affinity to FKBP, which sterically prevents extension of the  $A\beta$  fibril (Fig. 1).

Additional work is required to render the approach suitable for *in vivo* application. For example, in the human brain, the concentration of  $A\beta$  is less than 10 nM, so bifunctional inhibitors must have a smaller dissociation constant (that is, a stronger binding affinity) to induce inhibition. Gestwicki et al. achieved a 50 percent inhibitory concentration ( $IC_{50}$ ) of approximately 50 nM for compounds that recruit FKBP and block the formation of fibrils, resulting in the formation of small, amorphous aggregates of  $A\beta$ .<sup>1</sup> Under these conditions, the  $IC_{50}$  and the dissociation constant are similar. With additional effort, it should be possible to find small, bifunctional molecules that selectively cap the growing fibril by recruiting an endogenous protein and, in the process, lower the dissociation constant and  $IC_{50}$  to values below the nanomolar range.

Emerging evidence implicates the precursors of



**Figure 1. Inhibiting A $\beta$  Amyloidogenesis.**

A $\beta$  amyloidogenesis involves A $\beta$  aggregation into spherical structures and then into fibrils. The fibrils appear to grow most efficiently through the addition of monomeric A $\beta$ . When such fibrils are exposed to inhibitors with a high dissociation constant (weak binders), they continue to grow, albeit more slowly, when the inhibitor dissociates. There is some evidence that the plasticity of the amyloid structure allows the fibrils to grow even when inhibitors with a low dissociation constant are bound, owing to their small size in relation to the large protein surfaces involved in fibrilization. A recent study suggests a strategy to overcome these problems. Gestwicki et al.<sup>1</sup> have shown that small, bifunctional molecules such as chemically modified forms of Congo red (synthetic ligand for forkhead binding protein [FKBP]–Congo red conjugate [SLF-CR]) can bind a fibril or its precursor and then recruit an endogenous nonamyloidogenic protein — in this case, FKBP — thus, sterically hindering A $\beta$  amyloidogenesis. The right combination of a bifunctional small-molecule inhibitor and endogenous protein could result in much stronger binders and perhaps prevent the aggregation of A $\beta$  in the brain.

amyloid fibrils and, possibly, off-pathway amorphous aggregates in the gain of toxic function associated with neurodegeneration.<sup>4,5</sup> Even though fluorescence and turbidity measurements showed that the bifunctional A $\beta$  inhibitors used by Gestwicki and colleagues blocked amyloidogenesis, atomic-force microscopy studies revealed the build-up of spherical and amorphous aggregates in the presence of these inhibitors. Thus, blocking a later stage of A $\beta$  amyloidogenesis could actually be harmful to neurons. Gestwicki and colleagues therefore tested the ability of Congo red–based inhibitors with added FKBP to reduce the neurotoxicity of aggregates of A $\beta$  in vitro and found that by inhibiting A $\beta$  aggregation, their bifunctional compounds could indeed inhibit the death of cultured hippocampal neurons.

The authors went on to show that the potency of amyloidogenesis inhibition predicts the ability of

the compounds to inhibit neurotoxicity. Further elaboration of molecules of this type offers promise for inhibiting interactions between proteins associated with pathology and normal physiology.

From the Skaggs Institute of Chemical Biology and the Department of Chemistry, Scripps Research Institute, La Jolla, Calif.

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