New Cellular Models for Drug Discovery in Alzheimer's Disease

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Disclosure: The concepts outlined in this presentation are the subject of both issued and pending, US and foreign patents

It is currently thought that the dementia of Alzheimer's disease is due to the neurotoxicity of the deposits or soluble aggregates of the amyloid-β peptide (Aβ) found in the cerebral cortex of patients. As a result the search for therapies has been based on the development of agents which clear Aβ in mouse models transfected with mutant genes associated with the early onset forms of the human disease.

Aβ is produced in everyone during the processing of the amyloid precursor protein (APP). Mutations which lead to early disease include variants of APP and in one of the processing enzymes, γ-secretase. This enzyme is a heterotetramer of which two components, presenilin 1 and 2, are commonly mutated in families with a history of the early onset disease.

Even though these mutations are not found in patients with late onset disease, in order to facilitate drug discovery for the treatment of the disease seen in the elderly, investigators have developed mouse models which have been transfected with the mutant human genes identified in patients with the early onset disease.

In 221 trials of drugs identified in these mouse models, investigators have taken a variety of approaches for drug development including:

1. Clearing Aβ by immunological interventions
2. Prevention of the formation of Aβ by blocking γ-secretase.
3. Administration of antioxidants
4. Hormone modifications
5. Dietary modifications

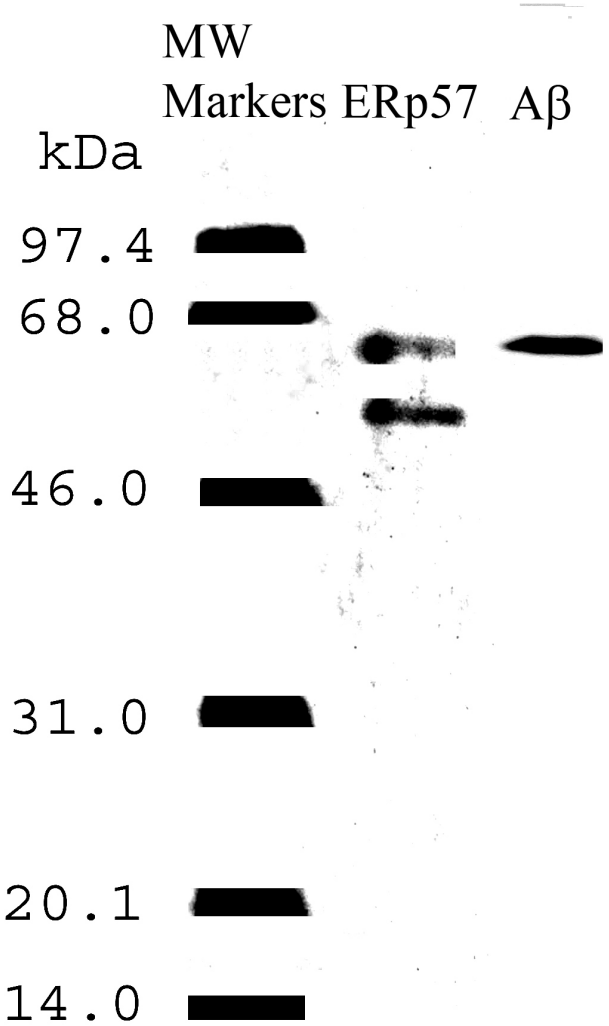
All of the disease modifying agents investigated in these trials have failed to show any benefit in elderly patients.

We began our studies with the question:

If Aβ is produced in everyone, why are deposits only seen in the brains of the elderly?

We proposed that normally Aβ is only present as a complex with two ER chaperones, ERp57 and calreticulin and is N-glycosylated. These modifications serve to keep it in solution.

And indeed this suggestion turned out to be correct.

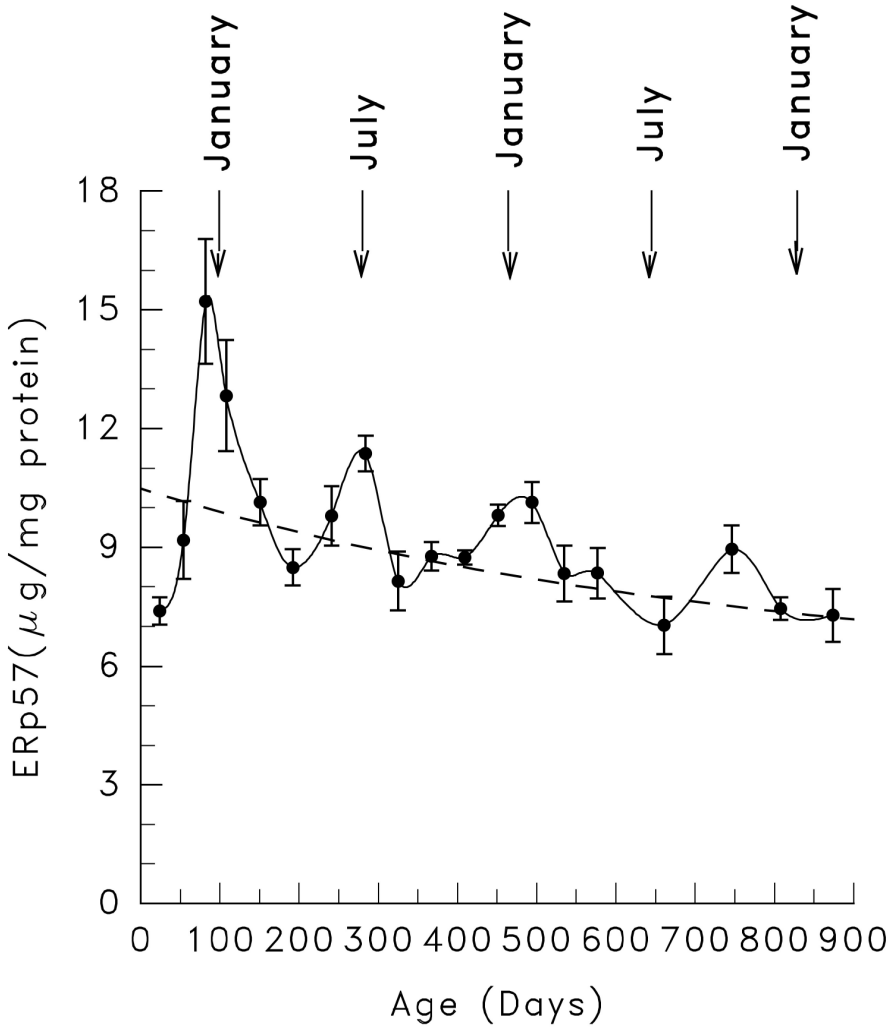


Western blot of Normal, Human CSF. Channel 2 - Antibody against ERp57; Channel 3 - Antibody against Aβ

Considering our results and the disappointing findings in the clinical trials, we proposed that Aβ deposits are only a biomarker for a decline in the capacity of the endoplasmic reticulum (ER) to catalyze the posttranslational processing of secretory and membrane proteins. **Including the synaptic, membrane proteins that are necessary for a functioning memory**

Furthermore, we have reported that the content of the ER chaperone, ERp57, which is a component of this complex, declines with age. Hence, one potential factor in the deposition of Aβ could be a decrease in the ER chaperone content.

Erickson *et al*. J. Gerentology 2006

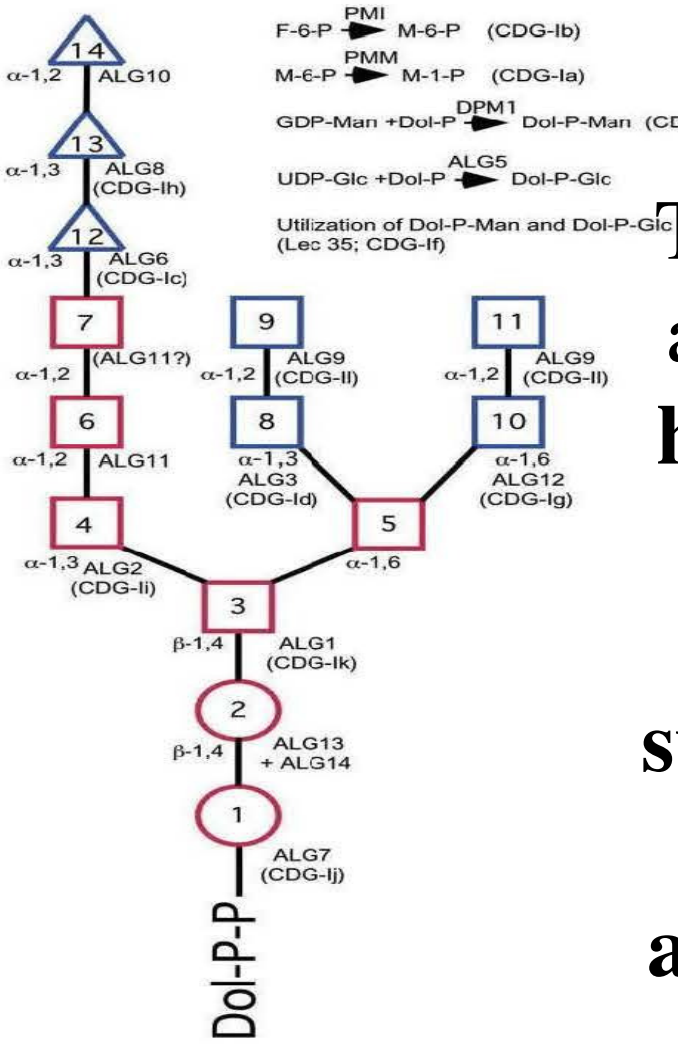


The effect of age on the ER content of ERp57 in rat liver

Erickson *et al*. J. Gerentology 2006

Finally, studies from other laboratories have suggested that the N-glycosylation pathway shows a marked decline with age. In this pathway an oligosaccharide is first synthesized bound to a high molecular weight lipid, dolichol. The carbohydrate complex is then transferred to the ε-amino group of a protein asparagine.

This hypothesis is supported on studies from many laboratories on the effect of age on the tissue content of dolichol. They have found that dolichol can increase as much as 5 to 10 fold with age. Since with age there is no increase in the synthesis of dolichol, these data suggest that its accumulation is due to a metabolic block in the first step in the synthesis of the oligosaccharide. This step is catalyzed by an enzyme designated as ALG7



The addition of each sugar is catalyzed by a series of unique monosaccharide transferases. These are highly conserved in both animals and fungi. **Furthermore, homozygous knocking out any of them is a lethal mutation! The increases in dolichol with age suggest that the primary defect in this pathway is a decline in the activity of the first enzyme in this pathway, ALG7**

Based on these observations it would appear that efforts to enhance the content of the ER chaperones and increase the activity of ALG7 may be promising targets for the identification of potential therapeutic agents to treat Alzheimer's disease in the elderly.

The usual approach for the development of agents which enhance the transcription of target proteins is to construct cell systems which have been transfected with luciferase attached to the promoter region of the gene for the target protein. Such constructs can be used for the rapid screening in microtiter plate readers of large libraries of potentially effective therapeutic agents.

A major shortcoming of these constructs is that recent studies in cell biology and biochemistry have demonstrated that the transcription and translation of genes for the synthesis of proteins are controlled not only by the promoter region, but also by a variety of other components of the cell, such as microRNA's, which are encoded in what in the past has been termed "junk DNA".

In order to identify agents which may affect these other regulators of transcription and translation, I have proposed to transfect the genes for fluorescent proteins, such as green fluorescent proteins (GFP) into the exons of the target proteins. Since GFP has a very compact structure, it has only a modest effect on the mature configuration of the target protein and therefore usually has no effect on the normal function of the cell. These constructs would still be controlled by the usual cellular components which regulate transcription and translation during the synthesis of the target protein and not just those factors which bind to its promoter region.

This approach to labeling proteins is widely used in cell biology. As a result the methodology is well developed and the reagents are also widely available. Furthermore, there are a large number of investigators who routinely produce such constructs. And there are also companies and academic groups that will produce them for a very modest fee (as little as $2000/transfection). **These fluorescent constructs would facilitate the rapid screening in microtitre plates of large libraries of drugs.**

Promising agents discovered in the high throughput screening could then be tested in animals in which the critical proteins have been knocked down by the administration of antisense oligonucleotides or transfection with conditional viruses containing the antisense sequence. The construction of such transfected animals is also very inexpensive. Along with the identification of the animals which have been successfully transfected and the expansion of a colony of these animal, the total cost is only about $50,000 per animal model.