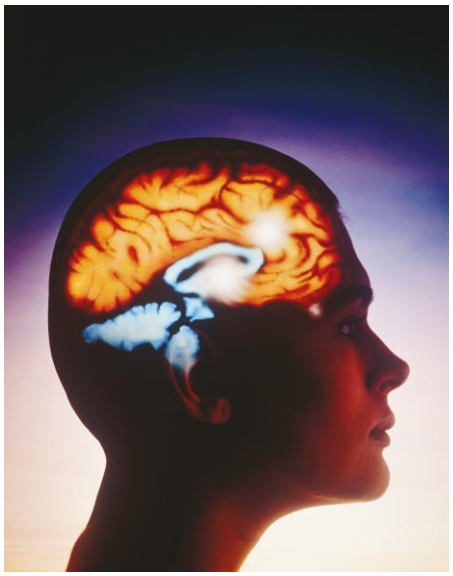
Untangling Alzheimer's disease with β-secretase inhibitors

Two inhibitors of the aspartic protease memapsin-2 (β-secretase), an enzyme closely involved in the development of Alzheimer's disease (AD), were recently developed at the Oklahoma Medical Research Foundation (OMRF; Oklahoma City, OK, USA) and the University of Illinois (Chicago, IL, USA)[1]. The new compounds, OM991 and OM992 (Fig. 1), pave the way for the development of new drugs to treat AD. 'The current inhibitors are peptidic and quite large. Their value is in demonstrating that the inhibitor design principles are applicable to memapsin-2. For real drugs, a new design that endows better pharmaceutical properties will be necessary,' comments Jordan Tang (OMRF), whose research team characterized memapsin-2 earlier this year[2].



Pathophysiology of Alzheimer's disease

Memapsin-2 is abundant in the human brain. It catalyses the rate-limiting step in the production of amyloid β-peptide (Aβ), a peptide that is deposited in plaques in the brain tissue and in the walls of cerebral blood vessels of people with AD. Abnormalities in the metabolism of Aβ occur early in the onset of AD and raised levels of the peptide are linked with a decline in cognitive function[3]. Moreover, raised Aβ levels seem to occur even before the appearance of the neurofibrillary tangles in the frontal cortex of the brain that are also characteristic of AD (Ref. 3). These tangles are the result of abnormalities in the conformation of the microtubule protein tau. They also occur in conditions and areas of the brain that are not associated with increased Aβ levels or AD. The peptide might therefore not be the direct cause of the tangles, even though it plays a central role in AD pathology[3].

Aβ is formed by the action of two proteases, a β-secretase and a γ-secretase, on the membrane-bound protein amyloid precursor protein (APP). The β-secretase generates the N-terminus of Aβ by cleaving APP at Met670/Asp671, while γ-secretase cleaves the protein to produce the C-terminus. As the γ-secretase cleaves either at Val711 or Ala713, the resultant Aβ is either 40 or 42 residues in length. The longer variety (Aβx-42) is more prone to aggregation, although both are deposited in the plaques associated with AD. Aβx-42 is also observed in the brains of individuals affected by Down's syndrome.

Tang's group finally identified the elusive β-secretase as memapsin-2, an enzyme whose sequence is in the expressed sequence tag database of human gene sequences. They cloned and sequenced the cDNA of the enzyme and revealed that it is similar to other aspartic proteases with the exception that it is membrane bound; memapsin-2 possesses an 80-residue C-terminal extension that comprises both transmembrane and C-terminal cytosolic domains. Tang and colleagues also confirmed that recombinant memapsin-2 hydrolyses APP and, when both the enzyme and APP are expressed in HeLa

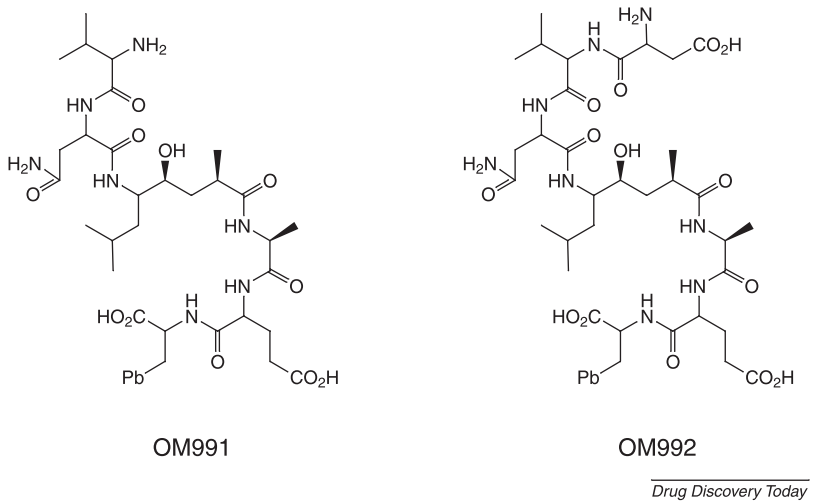


Figure 1. *Chemical structures of OM991 and OM992.*

1359-6446/00/$ – see front matter PII: S1359-6446(00)01531-2

cells, memapsin-2 cleaves the peptide at the β-secretase site within the cells.

Inhibiting memapsin-2

The group then began searching for specific inhibitors of the enzyme. Effectively, the group created a decoy to prevent the enzyme cleaving APP. 'OM991 and OM992 are analogues of the transition-state aspartic protease. In other words, we mimic the steric structure of the protease at the transition-state of its catalytic mechanism,' says Tang. 'The principle was first proposed by Linus Pauling and, in my lab in the 1960s, we showed that pepstatin was the transition-state analogue inhibitor for a pepsin-like enzyme. This work paved the way for the development of HIV protease inhibitors and, now, memapsin-2 inhibitors,' he explains.

As recombinant memapsin-2 only poorly hydrolyses the β-secretase site of wild-type APP, Tang and colleagues used a modified sequence from the same site of Swedish mutant APP to act as a template for the new memapsin-2 inhibitors[1]. This peptide is derived from APP β-secretase with the so-called 'Swedish mutation'. It is known to increase Aβ production in cells and has been shown to be a better substrate for mempasin-2. The resultant OM991 and OM992 were highly effective inhibitors of recombinant memapsin-2 and the group is now working on reducing the size of the inhibitor molecules, improving lipophilicity to aid movement across the blood–brain barrier, and increasing their specificity.

Future studies

Tang says, 'We are investigating new inhibitors derived from OM992, the most powerful of the two memapsin-2 inhibitors, to develop better properties and pharmaceutical potential.' The selectivity of any memapsin-2 inhibitor used therapeutically is especially important to ensure that other aspartic proteases in the body, such as cathepsin D, remain unaffected.

'Gamma-secretase is another major target for AD drug design', adds Tang. 'As there is no effective drug for AD, both γ- and β-secretase inhibitors should be developed, especially as we know that in other areas of medicine, such as hypertension, a single drug is not enough for patients with different responses to different drugs,' he predicts.

REFERENCES

1 Ghosh, A.K. *et al.* (2000) Design of potent inhibitors of human brain memapsin 2 (β-secretase). *J. Am. Chem. Soc.* 1222, 3522–3523

2 Lin, X. *et al.* (2000) Human protease memapsin 2 cleaves the β-secretase site of β-amyloid precursor protein. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1456–1460

3 Näslund, J. (2000) Correlation between elevated levels of amyloid β-peptide in the brain and cognitive decline. *J. Am. Med. Assoc.* 283, 1571–1577

Sharon Dorrell