in this way.' Studies have shown that increasing acetylcholine by inhibiting AChE can ameliorate the cognitive deficits in the early stages of AD [4].

Rivastigmine in action

There are around half a dozen AChE inhibitors on the market, including rivastigmine, which is sold under the name Exelon [5]. The drugs fall into three classes: tertiary amines, organophosphates and carbamates. Rivastigmine is a carbamate, and forms a covalent bond, which is slowly reversible, with AChE.

The Weizmann team performed kinetic studies of AChE with rivastigmine and found that the drug binds to AChE much more slowly and for a longer time period than expected, providing evidence of highly effective pharmokinetics.

X-ray crystallography determined why this is probably happening. Crystals of the fruitfly AChE, were soaked in rivastigmine. During binding, it was expected that rivastigmine would form a covalent bond and then split, with the leaving group washing into solution. The X-ray picture showed the leaving group was still bound to AChE. 'This is somewhat surprising,' says Sussman. 'It seems this causes the protein (AChE) to be distorted. So you've very slightly changed the 3D structure of the protein.'

'Most people would expect the leaving group to diffuse from the active site,' says Terrone Rosenberry, Professor of Medicine at the Mayo Clinic (http://www.mayo.edu). 'Instead, it is still sitting there at the active site, even though it is not linked to another part of the drug. So that's surprising.' Either the binding by the leaving group or the distortion of the AChE could explain why rivastigmine lowers the activity of AChE for such an extended period. 'It shows that rivastigmine might have better efficacy than these other drugs,' says Rosenberry.

Kinetic studies

Sussman says the kinetic study shows that rivastigmine might bind to AChE for tens of hours. Shutting down the activity of AChE can effectively increase the levels of acetylcholine in the early stages of AD when the neurons are not making enough of the neurotransmitter. 'When the disease progresses to a point that little or no acetylcholine is being made, then this is not a good drug,' he adds.

Rosenberry says that besides the information on rivastigmine, this study also shows some interesting science about the nature of leaving groups that stay attached to their target. 'The science is interesting because it could apply to other substrates and substrate analogues of AChE for which there is no crystal data.'

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Superpeptide to treat *Candida albicans*

Vida Foubister, freelance writer

A peptide analogue of α-melanocyte stimulating hormone (α-MSH) has been found to have potent antimicrobial activity against *Candida albicans*. This 'super' peptide killed nearly 100% of yeast cells in repeated experiments [1]. Its potency, coupled with the relative lack of toxicity of α-MSH peptides, suggest that this molecule holds the potential for use as a treatment for *Candida* and other infections in humans.

Candida species, including *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. kefyr*, *C. krusei* and *C. glabrata*, are a component of normal human flora. 'We only have trouble with it when it gets out of bounds,' said James M. Lipton, founder and director of Zengen (http://www.zengen.com), who have developed the peptide against candidal vaginitis.

'The use of peptides is a hot area,' says Richard Meagher, Director of the Sramek Center for Cell Engineering at Rush-Presbyterian-St Luke's Medical Center (http://www.cancercelltherapy.org/). 'You can use them to fight infection, [and] you can use them to fight tumors,' he states.

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Growing resistance

C. albicans, for example, is present at low numbers in the vagina of healthy women. But the overgrowth of this fungus can lead to vulvovaginal candidiasis, a condition commonly known as vaginal yeast infection and which affects 75% of women at least once in their lifetime. It also invades other tissues to cause infections that, when systemic, can be life threatening. This is an increasing problem in immunocompromized patients, including those suffering from HIV/AIDS or undergoing organ or bone transplants.

Azole drugs, such as fluconazole, are commonly used to treat *C. albicans* infections. However, there is evidence that these medications are becoming ineffective. 'The organisms have become resistant so that the success with conventional treatment is going down,' said Lipton. These drugs also have side effects in some individuals.

By contrast, peptide resistance is uncommon. In addition, α-MSH peptides have been found to have little or no toxicity *in vitro* or in preclinical studies [1]. This is thought to be because, in part, they hold a strong positive charge. 'These peptides are not going to be attracted to normal human cells, which are basically neutral in charge. They are going to be attracted to negatively charged microbes,' Meagher said. 'It's almost a targeting device.'

Peptide design

Scientists were originally interested in whether α-MSH (Fig. 1), like most anti-inflammatory agents, would reduce bacteria and fungal killing. 'To our surprise, it enhanced killing,' said Anna Catania, Professor of Endocrinology at the University of Milan (http://www.unimi.it). 'We never would have expected this peptide to have antimicrobial effects.'

α-MSH $Ac\text{-}Ser^{1}\text{-}Tyr^{2}\text{-}Ser^{3}\text{-}Met^{4}\text{-}Glu^{5}\text{-}His^{6}\text{-}Phe^{7}\text{-}Arg^{8}\text{-}Trp^{9}\text{-}Gly^{10}\text{-}Lys^{11}\text{-}Pro^{12}\text{-}Val^{13}\text{-}NH_2$

Drug Discovery Today

Figure 1. Structure of α-melanocyte stimulating hormone (α-MSH). Figure reproduced, with permission, from Ref. [1].

Catania and her colleagues then chose to investigate the antimicrobial activity of the C-terminal tripeptide Lys-Pro-Val (residues 11–13). This molecule, similar to α-MSH, has anti-inflammatory and anitpyretic effects, and it was found to have potent anti-infective activity against *Staphylococcus aureus* and *C. albicans* [2]. They also demonstrated that the candidacidal effects were mediated through increases in cyclic AMP, leading to the hypothesis that there is an unidentified MSH receptor in yeast.

Zengen has developed a molecule based on these results and found it to be effective against candidal vaginitis in clinical trials conducted in China. The molecule – CZEN002 – is composed of two tripeptides held together with a cysteine linker. This design was based on the knowledge that the simultaneous activation of two receptors increases the potency of ligands. CZEN002 is currently in a combined Phase I–II clinical trial for the treatment of vaginitis in the USA.

In their most recent study, the researchers – led by Catania – designed and synthesized 28 novel peptides to see if they could obtain a more potent molecule [1]. They also wanted to further elucidate the relationship between the structure of α-MSH and its antifungal activity. To do this, they focused on amino acids 6–13, which contain the invariant core sequence His-Phe-Arg-Trp (6–9) that is important for binding to known melanocortin receptors and the sequence Lys-Pro-Val (11–13) that is known to be important for antimicrobial activity.

Promising potency

One analogue in particular, peptide 19, in which Phe7 was replaced with D-Nal and Pro12 with Phe, was found to have pronounced candidacidal activity. These changes increased the hydrophobicity of the molecule but did not alter its net positive charge.

Peptide 19 killed 99.7± 0.4% of the *C. albicans* cells in repeated experiments and has also been shown to be effective against *C. krusei* and *C. glabrata*. 'Azole compounds act by disturbing the organism's membrane and they may interfere with metabolism,' Lipton said. 'We think our molecule works through a receptor. If this is true, it will be a truly novel way of controlling *Candida*.'

Work is currently under way to try and identify that receptor. In addition, Zengen plans to begin clinical trials to investigate the ability of this novel peptide analogue to treat vaginitis in Chinese women later this year. Although the initial clinical focus has been on topical treatments, the company intends to explore the use of these molecules for systemic infections as well. In addition, its researchers are exploring other uses for α-MSH, such as its protective effect in experimental heart transplantation [3].

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