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Drug designers seek a structural solution

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Agreement on the mechanism by which a putative prion-disease drug apparently blocks disease progression looks far from imminent, fear structural biologists. But, one has to wonder, does it really matter?

According to a German research group, quinacrine, a drug that temporarily blocks the symptoms of mad cow disease or new variant Creutzfeldt-Jakob disease (nvCJD), binds to a double-tyrosine motif in helix 1 of the aberrant prion protein, PrPSc. But the findings, presented at the *International Conference on Molecular Structural Biology* in Vienna, Austria (3–7 September 2003; http://pharmchem.kfunigraz.ac.at/icmsb/), run counter to data just published by a Swiss group, leaving researchers wondering just what quinacrine is up to.

NMR data

Data from nuclear magnetic resonance (NMR) spectroscopy reveal that when a peptide of PrPSc helix 1 is mixed with quinacrine, the drug binds to protein residues 149 and 150, both of which are tyrosines, says Stephen Schwarzinger, team leader at the University of Bayreuth, Germany (http://www.uni-bayreuth.de).

Although helix 1 is buried inside the bulk of the intact protein, Schwarzinger's group surmised that it might be the binding site of the drug because a number of the aromatic residues in the region are exposed to solvent. Also, there is evidence that helix 1 changes from an α-helical structure to a β-sheet during the change from cellular (PrPC) to disease (PrPSc) forms, and that inhibiting this transition might be important for slowing the disease, says Schwarzinger.

'Indeed we found that quinacrine binds with millimolar K_ds to this peptide,' Schwarzinger told *BioMedNet News* (http://news.bmn.com). 'We think the prion protein undergoes movement like breathing,' he said, explaining that a subdomain that includes helix 1 and β-sheet 2 becomes exposed during that movement, allowing the drug access.

Two are better than one

A group led by Fred Cohen at the University of California at San Francisco (http://www.ucsf.edu) found that the efficacy of quinacrine *in vitro* could be improved by linking two quinacrine molecules together, forming a compound called bis-quinacrine. The optimal linker distance between the two molecules corresponds to the distance between the double-tyrosine motif in helix 1 and a second double-tyrosine motif in β-sheet 2, says Schwarzinger. He thinks this agreement in spacing is further evidence that the native drug binds to helix 1 *in vivo*.

However, researchers at the Institute for Molecular Biology and Biophysics in Zurich, Switzerland (http://www.mol.biol.ethz.ch), found that the drug binds to a double-tyrosine residue in the C-terminal region of the full-length PrPSc protein. This domain lies on the exterior of the folded, intact protein.

'It is always dangerous to use peptides as a model system for proteins,' said Ralph Zahn, who led the work in Zurich. 'In the cell, we have a prion protein, not a prion peptide.' However, the two results do not totally contradict one another, says Zahn, because both groups find that the drug interacts with double-tyrosine residues at millimolar concentrations.

Important insights

Cohen, who's lab developed the bis-quinacrines and who has treated patients with quinacrine, says that although he agrees with Zahn that it is generally preferable to work with intact proteins rather than peptides, it is not yet clear which set of data is correct. In fact, he points out the drug could bind to another protein in the system with higher affinity. 'There is a long history in drug discovery of thinking that drugs work in one way only to find out much later that they work another way altogether,' said Cohen.

'We looked for quinacrine binding but only tested it at micromolar concentrations and didn't see anything,' he said. These two groups, however, noted that the drug is highly concentrated in membrane-bound compartments like the lysosome, which is where PrPSc also tends to aggregate. That insight, says Cohen, was crucial to Schwarzinger and Zahn's ability to locate the millimolar binding activity, and he credits both groups with having that important insight.

How valuable the binding site information will be remains unclear, says Cohen. 'I am a huge believer in structure-based drug design – I take it as a matter of faith that structure will help – but we improved the drug's efficacy by 10–20-fold with the bis compounds, without knowing structure,' he said. 'Maybe it could have been 500-fold with the structure, but who knows?'

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