INFLAMMATION

Free radicals on trial for microbicide: not guilty?

Current dogma states that reactive oxygen species (ROS) — also known as free radicals — produced during inflammatory action by phagocytic immune cells cause the death of pathogens. Furthermore, it is thought that oxidative stress can have a role in cancer, and autoimmune and neurodegenerative diseases, prompting the development of antioxidant drugs to block the effect of ROS. Now, research published in *Nature* casts doubt on whether ROS are solely responsible for killing microbes, indicating instead that proteases might also be involved.

White blood cells, such as neutrophils, have a crucial killing function in innate immunity: the production in phagocytic vacuoles of ROS, such as hydrogen peroxide, nitric oxide, superoxide and highly reactive hydroxyl radicals. These products of the enzyme nicotinamide-adenine dinucleotide phosphate (reduced) (NADPH) oxidase are thought to kill microbes efficiently. Proof of the importance of NADPH oxidase can be seen in patients deficient in the enzyme, who suffer from chronic granulomatous disease, often associated with life-threatening infections.

However, evidence is emerging that proteases in the phagocytic vacuole, activated by NADPH oxidase via the generation of a hypertonic, alkaline and potassium ion-rich environment, are responsible for microbial killing. Anthony Segal and colleagues showed that the large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) in the vacuolar membrane has an essential function in the microbicidal process. Two specific inhibitors of BK_{Ca} blocked NADPH oxidase-induced changes and alkalinization of the phagocytic vacuole, whereas a BK_{Ca} channel opener enhanced both changes. Microbial killing and digestion were abolished

CARDIOVASCULAR DISEASE

Ezetimibe explained?

Medicine has a long tradition of pragmatic empiricism, in which one does what works, regardless of understanding why it works. In the era of rational drug design, however, it is a surprise when new therapeutics make it through clinical trials without knowledge of their molecular targets. But such was the case for the cholesterol-lowering agent ezetimibe (Zetia/Ezetrol; Merck/Schering-Plough).

Ezetimibe was approved by the US FDA in November 2002 for the treatment of hypercholesterolaemia (alone and in combination with statins, which inhibit cholesterol synthesis). At the time, its mechanism of action was unknown. A recent paper in *Science* by Scott Altmann and colleagues from the Schering-Plough Research Institute now sheds light on this enigma by identifying a molecular pathway that ezetimibe affects.

Serum cholesterol levels are principally controlled by two organs: the liver, which synthesizes cholesterol, and the intestine, which absorbs dietary cholesterol. Acyl-coenzyme A:cholesterol acyltransferase (ACAT) is an enzyme involved in cholesterol absorption in the intestine, a process that is poorly understood. Schering-Plough, targeting ACAT for the development of a cholesterol-lowering drug, identified a class of compounds that inhibited cholesterol absorption in a cholesterol-fed hamster model. Experimental data, however, indicated that these agents acted upstream of ACAT. Nonetheless, ezetimibe emerged as a potent cholesterol-lowering agent.

So, if ACAT is not the target of ezetimibe, what is? To address this question, Altmann and colleagues carried out a genomic search to identify, from intestinal cells, rat transcripts that possessed features of a cholesterol transporter. Only one credible candidate emerged from this search: the rat homologue of the gene encoding human Niemann–Pick C1-like 1 (NPC1L1) protein. Analysis of the tissue distribution of NPC1L1 showed that expression is enriched in the small intestine.

The authors then investigated the *in vivo* role of NPC1L1 using mice engineered to lack the protein. Although otherwise phenotypically normal, NPC1L1-knockout mice absorbed just 31% of the amount of orally administered cholesterol that mice with functional NPC1L1 absorbed, indicating that the protein has a key role in cholesterol absorption from the intestine. And when the authors tested the effects of ezetimibe in normal and NPC1L1-knockout mice, they found that it reduced cholesterol absorption in mice with functional NPC1L1 to a level similar to that in untreated mice that lacked NPC1L1. NPC1L1-deficient mice, however, were completely insensitive to the drug. These data indicate that ezetimibe acts on an NPC1L1-containing pathway, and although the group was unsuccessful in detecting binding of ezetimibe to NPC1L1, it seems that the secrets of this drug's action are at last being revealed.

Daniel Jones

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when the channel was blocked, despite totally normal oxidase activity, phagocytosis and composition of granule enzymes.

Although the relative contributions of ROS and proteases to microbial killing in phagocytes is still in the balance, ROS are produced in all cells and can damage DNA, proteins and lipids. The success of anti-oxidants for disease states will ultimately be determined in the clinic, and even though there is good reason to be optimistic on this front, the results remain to be seen.

Melanie Brazil

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VIRTUAL SCREENING

Gliding to success

When high-resolution structural data for a protein target are available, computationally 'docking' molecules from large databases into the protein structure and scoring their calculated binding affinities can be a valuable aid for enriching the libraries used in screening assays with molecules that are likely to be active. The accuracy of the docking and scoring is crucial to the success of such approaches, and two recent papers in the *Journal of Medicinal Chemistry* describe a new docking methodology — named Glide — that can outperform methods that are generally viewed as representing the current state-of-the-art in docking, such as GOLD and FlexX.

A key problem in docking is that assessing the interaction of all possible conformations, orientations and positions of a given ligand with even one rigid model of the protein receptor is expensive in terms of computational time, and so becomes impractical in database screening unless the library is small. But if all such interaction possibilities are not assessed, important possibilities could be missed.

As described in the first paper, Glide aims to address this issue by approximating a complete systematic search of the conformations, orientations and positions of the docked ligand, allowing large libraries to be screened in a reasonable period of time. This is achieved by using a series of hierarchical filters so that the most computationally expensive calculations are performed only on a very small fraction of the possible docking solutions for each of the compounds in a large database screen. To boost accuracy while restraining computational costs, the authors also used a strategy in which only a small fraction of the hits identified by Glide were redocked using a more computationally expensive 'extra-precision' version of the program.

Assessment of the docking accuracy of Glide by redocking the ligands from 282 protein–ligand complexes in the Protein Data Bank into the corresponding proteins showed that Glide was nearly twice as accurate as GOLD, and more than twice as accurate as FlexX for ligands having up to 20 rotatable bonds. So, how would this translate into an ability to enrich screening collections with active compounds?

To provide some insight into this question, and as reported in the second paper, the authors tested the ability of Glide to identify known active compounds in a database of druglike 'decoy' ligands selected to be representative of a typical corporate compound collection. Nine widely differing protein targets were assessed, including thymidine kinase, the oestrogen receptor and HIV protease. Glide was successful in yielding 'enrichment factors' of at least 10 — which corresponds to all the active compounds being in the top 10% of the binding scoring list — for five of the nine targets, and more than five for all but one. Furthermore, comparison with previously published data on thymidine kinase and the oestrogen receptor found that Glide performed better than GOLD 1.1, FlexX 1.8 or DOCK 4.01. It will be interesting to see how successful Glide is in real-life screening situations, and in dealing with the particularly challenging issue of protein flexibility.

Peter Kirkpatrick

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