Innovations

Getting rid of radicals

MetaPhore Pharmaceuticals, Inc.

Chemistry & Biology December 1999, 6:R345–R346

1074-5521/99/$ – see front matter

Modern molecular biology is geared towards genes and proteins, with small-molecule chemistry coming in a distant third. Small molecules are important as drugs, but largely ignored in understanding how the cell works.

The revival of small molecules started with the discovery that endothelium-relaxing factor, so important for controlling blood pressure, was nitric oxide (NO). With a string of recent discoveries, superoxide ($O_2^{\cdot -}$) has now taken center stage. This oxygen radical has been implicated in a long list of normal and disease processes, including reperfusion injury (when a blood supply is re-established following surgery, a heart attack, or stroke), neurodegenerative and autoimmune diseases, and inflammatory and mitogenic signaling. Getting rid of superoxide has become a major priority.

A low level of superoxide is constantly generated by aerobic respiration. The electron-transport chain of mitochondria, which is meant to escort four electrons to molecular oxygen to form water, occasionally leaks a single electron. "It's like a wire with insufficient insulation," says Irwin Fridovich of Duke University, Durham, North Carolina.

In 1969 Fridovich and Joe McCord (University of Colorado Health Sciences Center, Denver) discovered the body's primary mode of defense against this leakage: superoxide dismutase (SOD). SOD converts superoxide to hydrogen peroxide (H_2O_2) and molecular oxygen. It is remarkable for its use of electrostatic guidance of substrates to exceed diffusion-limited catalytic rates. But as a treatment SOD was found wanting — it was unstable, didn't penetrate into cells, and provoked an immune response. Now a group of companies, including MetaPhore Pharmaceuticals, Inc. (St Louis, Missouri), is trying to mimic the SOD enzyme with cell-permeable small molecules. MetaPhore's human testing is only scheduled to start late in 2000, but already, says Fridovich, "they've had good success. The principle has been proven."

Enzyme design

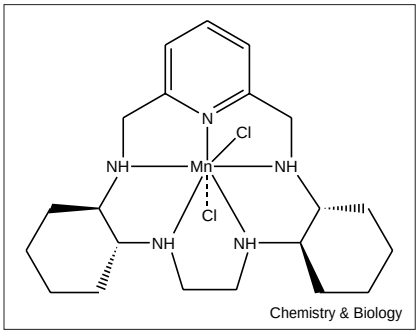
Metals are good at doing redox chemistry, but indiscriminate redox chemistry by free metals is very toxic to the cell. Therefore, says MetaPhore's vice-president of research Dennis Riley, "if you're going to have a metal as a redox drug you need to have it in a stable ligand. The way to do it is the way nature does it — with a macrocycle."

Small molecules that mimic enzymes can destroy disease-causing radicals.

Riley started with manganese, which is far less toxic than copper or iron, the other two metals used in native SODs. In his first series of macrocycles there was just one structure — a cyclic penta-aza compound — that was both catalytically active and reasonably stable (Figure 1).

Improving on the initial compound took an understanding of the reaction mechanism. Riley found that the rate-limiting step of the reaction cycle was the oxidation of the Mn(II) state to the Mn(III) state. In the Mn(II) state the five nitrogen ligands were in a single plane, with two additional Mn ligands above and below this plane. But in the Mn(III) state one of these axial ligands was replaced by one of the five nitrogens.

Figure 1



One of the macrocycle SOD mimics under investigation at MetaPhore. The two cyclohexano groups (bottom right and left) keep the molecule in a bent conformation that mimics the Mn(III) state, therefore speeding the rate-limiting Mn(II) to Mn(III) oxidation reaction. The curvature is robust enough to withstand the planar-favoring effects of the pyridine group (top), which was added to increase stability.

Riley realized that in an efficient enzyme, "you don't have time for the ligand to change shape. You have to hold the precursor in a geometry that is the same shape as the product." He therefore added substituents to hold the Mn(II) compound in a folded conformation, with one of the nitrogens out of the plane, ready for the conversion to the Mn (III) state. "Our compound behaves like an enzyme — it holds a ligand in a pre-organized state that it may not otherwise favor," says Riley. "They're truly little synthetic enzymes. They're just not made by transcription of DNA."

No room at the inn

The rational design process was a success, but the project was fighting for the attention of Riley's employer, the Monsanto Company (St. Louis, Missouri). Monsanto's pharmaceutical subsidiary, Searle, had several potential blockbusters on its hands, including the now highly successful Cox2-specific anti-inflammatory drug Celebrex. With the stakes in this market so high the company was, according to Riley, "shooting for the moon. They were trying to do everything in parallel, so there wasn't a way to do our program."

Monsanto offloaded the SOD technology to a small company called MetaPhore, which was already looking for metal chelates to treat the iron-overload disease hemachromatosis. "I was quite shocked and demoralized," says Riley. But Monsanto retained only a few royalties and the right of first offer in any buy-back deal, so in the end, says Riley, the freedom turned out to be "so much the better for us."

Further improvement of MetaPhore's lead compound has relied on knowledge of the reaction mechanism and a resulting computer model, says Riley. "Without it there was no way we would have had enough chemists to test a random empirical series."

The end result is a compound with a catalytic rate constant that exceeds 10^9 M^{-1} s^{-1}, which is ten times faster than the original Mn-based protein. "You need a very fast catalyst to interfere with the chain reactions started by superoxide," says Riley. With that in hand, Riley is ready for clinical trials.

So many diseases, so little time

Soon after its discovery as a byproduct of oxidative metabolism, superoxide began turning up in many biological systems: as a product of NADPH oxidase in phagocytes, which use a burst of superoxide to help kill bacteria; in signaling cascades involving NFκB in immune cells and ras in cancer cells; and after reperfusion or brain excitotoxicity when metabolism is suddenly resumed or increased.

Thus the list of diseases that may be treatable with SOD mimics now includes heart attacks, stroke, autoimmune diseases (such as osteoarthritis), neurodegenerative diseases (including Alzheimer's and Parkinson's) and aging.

MetaPhore is in the process of choosing its disease target. The company's current leads are not orally bioavailable, so it may decide on an accessible target like radiation-treatment-induced injury in cancer, which is an early indication for the compounds of Eukarion Inc. (Bedford, Massachusetts). Eukarion is also interested in neurodegenerative diseases, as their compounds reach the central nervous system. In these longer-term applications there will be more concern about immune suppression, although Eukarion vice president of research Susan Doctrow says her company has not seen signs of immune problems in animal models.

My radical is fiercer than your radical

Eukarion started with Mn salen compounds made by Eric Jacobsen (Harvard University, Cambridge, Massachusetts) as catalysts for asymmetric epoxidation. The compounds already had SOD activity, but the company modified them to increase catalase activity — the ability to break down hydrogen peroxide. "We believe the catalase activity in some instances is more important," says Doctrow. But Riley says "the only time [hydrogen peroxide] is a real problem is when you have superoxide around," because the superoxide reduces Fe(III) to Fe(II), releasing the iron from storage sites so that it can react with hydrogen peroxide and produce hydroxyl radicals.

John Groves at Princeton University (Princeton, New Jersey) has yet another target. Peroxynitrite ($ONOO^-$) is formed extremely rapidly when superoxide combines with NO. In trying to destroy superoxide before it combines with NO, "you're trying to buck against a very fast reaction," says Groves, whose peroxynitrite scavengers are being developed by Inotek Corporation of Beverly, Massachusetts. "It's not clear how many reactions superoxide does by itself," he says. "It could be that peroxynitrite is a major part of the story. There's some sorting out to be done."

As the companies fine-tune their respective compounds, that sorting out will become easier to do. Numerous animal tests of SOD mimics have been published, but Riley says the latest tests by MetaPhore made it into *Science* (**286**, 304–306 (1999)) because "it was the first time that a molecule was a totally selective SOD catalyst. And by eliminating the superoxide we eliminated the production of pro-inflammatory cytokines. Providing that link that superoxide is directly responsible for turning on these pro-inflammatory cytokines is very important."

A similar analysis should be possible for each damage reaction. "Armed with these compounds one can begin to sort out how much of the superoxide damage is through the peroxynitrite reaction," says Groves. "Just as important as the potential therapeutic applications of these agents, they're certainly going to be very important in deconvoluting the very complicated effects of [different reactive chemicals] in the cell."

A simple target

Fridovich has his own commercial interests — his porphyrin-like SOD mimics are being investigated by Aeolus Pharmaceuticals, Inc. (Research Triangle Park, North Carolina) — and he thinks that companies making SOD mimics have chosen their target well. "You're mimicking the reaction catalyzed by an intensely simple enzyme," he says. With only two reactants, "the catalyst is just taking the electron from one to another. If it was a more complicated organic reaction it would be hopeless. We have a particularly easy target to mimic."

If the chemistry is easy, perhaps it can be tailored to cure the ultimate modern obsession: aging. "Everybody is very well aware of the potential of free radicals in aging," says MetaPhore's director of biology Daniela Salvemini. As a company, she says, "you have to have priorities. Aging for now is not one of ours." But sooner rather than later, one would suspect, the aging specter will arise. After all, as Riley says, "these free radical reactions are the death of us."

William A. Wells
1095 Market Street #516, San Francisco, CA 94103-1628, USA; wells@biotext.com.