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Biomimetic Ion Chelators - Potential Therapeutic Agents and Diagnostic Tools

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Natural iron-carrying molecules (siderophores) are excreted by microorganisms for sequestering the essential iron ions from the environment; they are recognized by specific receptors on the membranes and are used for the insertion of these ions into the cells, where the iron 'cargo' is delivered and the siderophores are excreted to bring in more iron. For several years we have been involved in the synthesis of mimics to several families of the natural siderophores, and were able to demonstrate that properly designed synthetic siderophores are taken-up by bacteria and fungi membranal receptors and transport systems in a comparable manner to the natural compounds. Most significant and very surprising was the observation that some of the synthetic analogs were highly species specific, in some cases even more than their natural counterparts.

The modified skeleton of the biomimetic analogs enables the attachment of functional moieties. Their ability to penetrate into the cells is being used for preparing hybrids of siderophores and fluorescent markers, which are utilized as diagnostic tools, emitting fluorescent 'signals' to indicate the presence of particular microbes. It also contributes to the creation of new generations of drugs and prodrugs, which are inserted into cells via the natural transport systems. Only inside the cells the drugs are released by the action of enzymes. Thereby we bypass the problem of drug resistance. Attaching hydrophobic sidechains to the analogs allows them to cross the blood-brain barrier, while protecting the brain from damage caused by free radicals. Results of research supporting the latter considerations are presented below.

Other recent highlights of the research in the field of biomimetic siderophores are the following:

1. Synthesis of hydrophobic ferrichrome analogs that turn hydrophilic inside the cells and are able to sequester iron very efficiently. These compounds are envisioned to pave the route for the development of drugs against malaria.

2. Synthesis of compounds that mimic the active site of the peroxidase enzyme that show catalytic activity.

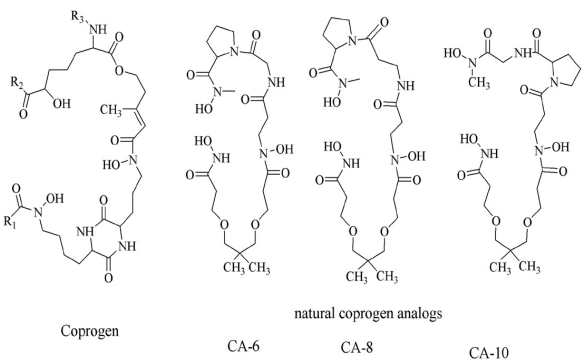

Fig. 1 *The molecular structure of the natural siderophore coprogen and three of its synthetic analogs*

Synthesis and biological evaluation of lipophilic iron chelators as protective agents from oxidative stress

The damaging effect of excess iron has been correlated to oxidative stress through the classical Fenton reaction where Fe (II) ions oxidize H_2O_2, leading to the generation of hydroxyl radicals, which are highly cytotoxic. The search for chelators that penetrate the blood brain barrier and reduce the free iron pool, thereby dropping the level of Fenton reaction, is an objective of many research groups throughout the world. The major drug used currently to treat iron overload is DFO (desferrioxamine), which is a natural iron chelator (isolated from *Streptomyces pilosus*). Although it has high affinity for Fe (III) ions, its slow onset of action, poor cell permeation, prolonged parenteral administration

and extensive dosages have prompted search for more effective iron chelators.

We have synthesized a novel class of low molecular-weight lipophilic iron chelators (Fig. 1) and checked their ability to exert protective effect in oligodendrial cells that were exposed to oxidative stress (Fe (II) and H_2O_2). We have found that all derivatives are capable of crossing the blood brain barrier and compete successfully with DFO. Moreover, one of the analogs (CA-6) has exhibited a 25-fold higher activity in comparison to DFO.

The protective ability of the chelators was examined by using a cell line of oligodendroglia origin (OLN 93) as a model for neural cells. In these experiments, OLN 93 cells were seeded in 96-well polyethyleneimine pre-coated plates, and after 24 hours, were further incubated for 3 hours with the synthetic analogs at various concentrations. A Fenton reaction was initiated and after two hours the reagents were removed. The cell survival was measured by a neutral-red assay, where the survival rate is directly proportional to lysosomal dye uptake as measured by the absorbance at 550 nm.

The results indicated that all analogs exert some protective effect as seen by increasing the overall survival rate of cells treated with H_2O_2 and Fe (II). Analog **CA-6** has showed the highest protective effect (93% survival rate). Additional experiments using this analog revealed apparent protection already at a concentration of 40 μM, and full protection at a concentration of 200 μM (Fig. 2). We also compared the activity of **CA-6** with that of DFO and found that, at the same concentration, the synthetic compound fully protects the cells from oxidative stress, DFO has only a negligible effect. We attribute this difference to the lipophilic character of the synthetic analog in comparison to the relatively hydrophilic character of DFO that enables better crossing of the BBB.

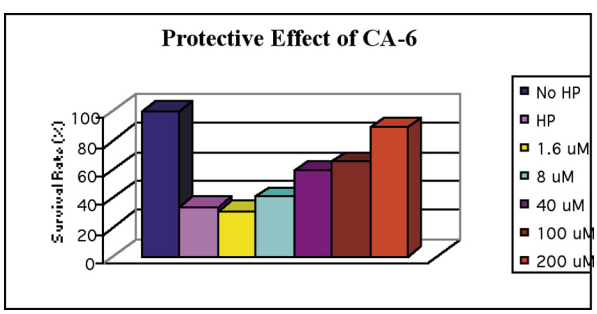

Fig. 2 *Survival rate of OLN cells after oxidative stress at different concentrations of CA-6. SEM<5% of the value (n=7)*

Selected Publications

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Synthesis and Biological Evaluation of Lipophilic Iron Chelators as Protective Agents From Oxidative Stress

Yavin, E. Raghavendra VK, Gil S. Arad-Yellin R. Yavin E. and Shanzer A. (2003) J. Am. Chem. Soc. Submitted for publication

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