An obstacle faced by both mimetics and enzymes is the unfortunate sharp bell-shaped dose-response curves seen when SOD activity is used to inhibit many superoxide-mediated phenomen,a such as reperfusion injury [2]. The behavior appears to be due to the ability of superoxide to initiate the chain reaction of lipid peroxidation and, paradoxically, to terminate the same process. Accordingly, a single optimal concentration of SOD activity exists for any pathological situation, reflecting a compromise that limits initiation events while still allowing significant termination to take place. The concentration of SOD activity is critical only while substantial lipid peroxidation occurs. Healthy cells can tolerate a range of SOD concentrations with few ill effects, but whether they fare better [3] or worse [4] when challenged by oxidative stress depends on precisely how much SOD is expressed, as seen in studies of transgenic mice exposed to ischemia/reperfusion. More is not always better, and sharp bell-shaped dose response curves are a pharmacological nightmare.

The questions of where superoxide is overproduced in a particular disease state and whether the SOD or mimetic has access to that location lead one to speculate that we might ultimately need a variety of SOD-like drugs with properties tailored to specific applications. In inflammatory diseases, the primary sources of superoxide production are activated neutrophils, resulting in free radical attack from outside the cell; in diseases of ischemia and reperfusion injury the superoxide is largely generated by mitochondria within the cell. Thus, it could be that enzymes are best suited for anti-inflammatory applications [5], whereas the mimetics, with access to the mitochondria, might be best suited for the treatment of reperfusion injury [6].

A third approach that has received little attention is drugs that can upregulate the body's production of its own antioxidant enzymes.

References

1 Day, B. J. (2004) Catalytic antioxidants: a radical approach to new therapeutics. *Drug Discov. Today* 9, 557–566

2 Nelson, S. K. *et al.* (1994) The toxicity of high-dose superoxide dismutase suggests that superoxide can both initiate and terminate lipid peroxidation in the reperfused heart. *Free Radic. Biol. Med.* 16, 195–200

3 Chen, Z. *et al.* (2000) Overexpression of CuZnSOD in coronary vascular cells attenuates myocardial ischemia/reperfusion injury. *Free Radic. Biol. Med.* 29, 589–596

4 Levy, R. *et al.* (2002) Ischemic reperfusion brain injury in fetal transgenic mice with elevated levels of copper-zinc superoxide dismutase. *J. Perinat. Med.* 30, 158–165

5 Gao, B. *et al.* (2003) Synthesis and anti-inflammatory activity of a chimeric recombinant superoxide dismutase: SOD2/3. *Am. J. Physiol Lung Cell Mol. Physiol* 284, L917–L925

6 Sheng, H. *et al.* (2002) Effects of metalloporphyrin catalytic antioxidants in experimental brain ischemia. *Free Radic. Biol. Med.* 33, 947–961

Joe M. McCord
Webb-Waring Institute for Cancer, Aging, and Antioxidant Research
University of Colorado Health Sciences Center
4200 E. Ninth Ave., Box C-321
Denver, CO 80262, USA
email: joe.mccord@uchsc.edu

Drug discovery in the HIF pathway: where do we go now?

In the USA nearly two-thirds of all deaths are attributed towards heart disease, cancer, cerebrovascular disease and chronic lung disease. Reduced oxygen levels (hypoxia) and deregulation of HIF, a hypoxia responsive transcription factor, have been shown to constitute a major component of these disease states [1]. Tremendous advances over the past few years have uncovered a family of hydroxylase enzymes at the centre of HIF regulation. This family of enzymes adds oxygen to key amino acid residues in HIF, playing an important role in sensing oxygen levels and regulating the activity of the HIF proteins [2].

In a recent article in *Drug Discovery Today*, Hewitson and Schofield [3] provide a timely review outlining our current understanding of the regulation of HIF by the HIF hydroxylases. The authors provide a balanced analysis of the opportunities and possible limitations facing the pharmaceutical industry wishing to develop therapeutic interventions for HIF.

Selecting the most appropriate point of the HIF pathway to target for drug development will be vitally important for achieving a successful clinical outcome. One would initially assume that the HIF hydroxylase enzymes would be that appropriate target. Hewitson and Schofield [3] suggest that targeting solely the HIF hydroxylase enzymes might prove technically difficult, essentially due to the similarity of the enzyme's active site with other known hydroxylase enzymes, such as those involved in collagen biosynthesis and DNA repair.

Instead, they suggest that it could be more advantageous to target the substrate-binding site located within the HIF protein. However, the disruption of protein–protein interactions has not been generally thought of as a suitable molecular target for drug development by the pharmaceutical industry. The reasoning behind this has been that small molecules are less likely to be effective in disrupting complex interactions between two proteins, which normally occurs over numerous interfaces, unlike inhibiting an enzyme by occupying its enzymatic site. In the current era of HTS this might no longer be a dilemma. For example, recent small-molecule inhibitors that successfully target the dimerization interface of the MYC and MAX transcription factor proteins have been discovered using HTS [4].

Over the past few years, the rapid progress made in understanding the

1359-6446/04/$ – see front matter PII: S1359-6446(04)03210-6

basic molecular mechanism of HIF regulation has provided exciting therapeutic opportunities. Whether these findings can now lead to the development of useful therapeutic interventions will depend largely on collaborative investigations between basic laboratory and clinical researchers.

References

1 Semenza, G.L. (2000) HIF-1 and human disease: one highly involved factor. *Genes Dev.* 14, 1983–1991

2 Lando, D. *et al.* (2003) Oxygen-dependent regulation of hypoxia-inducible factors by prolyl and asparaginyl hydroxylation. *Eur. J. Biochem.* 270, 781–790

3 Hewitson, K.S. and Schofield C.J. (2004) The HIF pathway as a therapeutic target. *Drug Discov. Today* 9, 704–711

4 Berg, T. *et al.* (2002) Small-molecule antagonists of Myc/Max dimerization inhibit Myc-induced transformation of chicken embryo fibroblasts. *Proc. Natl. Acad. Sci. USA* 99, 3830–3835

David Lando
dl280@cam.ac.uk

Opening the bottlenecks

Glenn E. Dale, Morphochem AG, Schwarzwaldallee 215, CH-4058, Basel, Switzerland; e-mail: glenn.dale@morphochem.ch

The Protein Expression conference held 28–29 April 2004 in London, UK, brought together around 100 delegates from academia and the pharmaceutical industry to address the major issues in protein production. Much of the conference was devoted to the expression of soluble proteins for crystallography, with sessions describing high-throughput techniques and alternative expression systems and analysis. The importance of structural biology research has been highlighted in the past few years, not only as an integral part of drug discovery programs in the pharmaceutical industry, but also through the efforts in academia and the many structural genomics programs that have been established. Statistics arising from the various structure-genomics programs reveal variable success rates from the cloned protein to the structure determination [1]. The expression of a protein has a direct impact on the successful application of crystallography. If a protein can be expressed in a soluble form and purified as a monodisperse species then there is a 70% chance of obtaining diffraction quality crystals, whereas proteins that are aggregated or polydisperse in solution have <10% chance of crystallizing [2].

High throughput and automation

Jean-Dominique Guitton (Aventis; http://www.aventis.com) began the conference by describing the road towards the industrialization of protein structure determination. He pointed out that there were 28 different structural genomics initiatives, however the success rate from cloned gene to solved structure was, on average, less than 5%. He described the main bottlenecks in the process as protein expression and the crystallization, whereas the purification, data collection, data processing and structure refinement processes have generally been industrialized. He presented a robotic platform for vector construction (with a throughput of 90 constructs per month) and a robotic solution to performing high-throughput expression trials in *Escherichia coli*. He stated that, using a parallel approach of multiple constructs and expression conditions, they were able to reduce the time for structure determination from >14 months on average to five months.

Konrad Büssow (Protein Structure Fabrik in Berlin; PSF, http://proteinstructurefabrik.de) presented a cost effective approach to high-throughput protein production for structural analysis. PSF developed a method for parallel expression and purification of recombinant proteins with a hexahistidine tag (His-tag) or glutathione S-transferase (GST)-tag from bacterial expression systems. Proteins are expressed in 96-well microplates and are purified by a fully automated procedure on a pipetting robot. Up to 90 µg of purified protein can be obtained from 1 ml microplate cultures. Grant Cameron (NextGen Sciences; http://nextgensciences.com) described the Expressionfactory™, an instrument that automates the cloning, expression and purification within a single platform. When combined with the proprietary software the Expressionfactory™ enables the parallel exploration of different constructs, host cells and growth conditions to optimally produce the desired protein.

Angelo Gunasekera (Abbott; http://www.abbott.com) described an approach where the gene of interest was cloned into *Bsa*I site of a vector (IISC vector system) and how this approach was used for expression of essential *Streptococcus pneumoniae* genes. They tried several *E. coli* host cells with each

1359-6446/04/$ – see front matter PII: S1359-6446(04)03203-9