NEURODEGENERATIVE DISEASES



The amount of iron in the brain increases with normal ageing, but elevated iron levels are also associated with several neurodegenerative diseases, including Parkinson's disease. Reactive ferrous iron (Fe²⁺) can participate in the generation of celldamaging free radicals, so it has been hypothesized that higher iron concentrations could play a part in the neuronal loss observed both in old age and, more drastically, in many diseases. However, as with many of the other observed biochemical and pathological changes that accompany neurodegenerative diseases, there is debate as to whether increasing iron levels themselves cause cellular damage, or are simply a consequence of damage caused by other factors. Convincing evidence for the detrimental effects of elevated iron in an acute animal model of Parkinson's disease is now presented in a study published in the March 27th issue of Neuron, in which Kaur et al. show that brain damage is diminished by reducing iron levels.

The authors used both transgenic and pharmacological methods to sequester iron in mice that were exposed to one or more doses of the neurotoxin MPTP, the standard method for producing animal models that mimic many of the signs of Parkinson's disease. In the transgenic approach, the heavy subunit of human ferritin was selectively expressed in dopaminergic neurons under the control of a tyrosine hydroxylase promoter. Ferritin converts harmful ferrous iron to unreactive ferric iron (Fe3+), which it sequesters in large quantities. The presence of ferritin greatly attenuated the loss of the vulnerable dopaminergic neurons in the substantia nigra, as assessed by stereological cell counts of tyrosine-hydroxylase-positive neurons seven days after MPTP administration. It also led to partial reverses in the increase in reactive oxygen species and decreases in glutathione levels seen after MPTP administration, changes which are also characteristic of Parkinson's disease brains.

A second iron-removing strategy - dosing with the metal chelating antibiotic 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol) — was also shown to attenuate the MPTPinduced cell loss and detrimental

INFLAMMATION

Promise for glycolysis inhibitor

Inflammation is part of the normal response to infection or injury, but prolonged inflammatory activity can cause diseases such as arthritis or Crohn's disease. In a study published in the March 7th issue of Cell, scientists report that inflammation can be prevented in mice by blocking the glycolytic metabolic pathway in macrophages and neutrophils used in hypoxic, or low oxygen, conditions.

Part of the inflammatory response involves the massive migration of these immune cells out of circulation to the site of tissue injury. One of the signals that guides the cells is the hypoxia that arises when the blood vessels at the wound site are disrupted. A protein called hypoxia inducible transcription factor- 1α (HIF1 α) is known to be essential for hypoxia-induced increases in glycolysis in some tissues, enabling cells to function in damaged

tissues by utilizing the glycolytic pathway to generate ATP.

The authors created a targeted deletion of the HIF1 α transcription factor in macrophages and neutrophils, and under hypoxic conditions the cellular ATP pool in these HIF1α-deficient cells was drastically reduced, confirming that HIF1 α is essential for the regulation of glycolytic capacity in these cells. The metabolic defect also renders these cells incapable of aggregation, motility and invasiveness, and reduces their ability to kill bacteria, all crucial components of an effective inflammatory response. When mice lacking HIF1 α were subjected to an arthritis-inducing stimulus, they did not develop joint swelling or other inflammatory symptoms. The authors also showed that the loss of Von Hippel Lindau, an HIF1α negative regulator and tumour suppressor, causes a hyperinflammatory

response. These data indicate that HIF1 α lies in a crucial position in the inflammatory pathway and that appropriate agonists or antagonists could either boost or block inflammatory activity, respectively. Interestingly, the loss of HIF1 α compared with the loss of vascular endothelial growth factor, a downstream target of HIF1 α , results in somewhat different phenotypes, indicating that the functions of the two molecules only partially overlap.

A large number of HIF1 α inhibitors are being developed for the treatment of cancer, as high levels of HIF1α expression are seen in some tumours and because of HIF1a involvement in angiogenesis. These drugs might turn out to have an even bigger market in treating inflammation and arthritis because they would have an advantage over present treatments, as they can completely block tissue invasion by inflammatory cells in animal models.

Melanie Brazil

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biochemical changes. Clioquinol chelates both ferrous and ferric iron and is already in Phase II clinical trials for another neurodegenerative disease, Alzheimer's disease, in which its metal-chelating properties are thought to inhibit β -amyloid accumulation. In this study, clioquinol was shown to protect neurons after both acute and chronic (five day) MPTP insults. Both the ferritin-expressing and clioquinol-treated mice were reported to show less decline in motor activity than normal animals following dosing with MPTP.

With the identification of ferrous iron as a causative element in the pathological progression of Parkinson's disease, the search is on for agents that can achieve the delicate balance of sequestering excess reactive iron in vulnerable regions of the brain, without damaging the many systems that rely on normal iron levels for their functioning.

Adam Smith

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THERAPEUTIC PROTEINS

Glycoengineered to last longer

Nobody enjoys having an injection. Spare a thought, then, for patients that suffer from chronic anaemia, who require frequent injections of recombinant human erythropoietin (rHuEPO) to boost their production of red blood cells. Some relief might be afforded by the recent development of an analogue of rHuEPO, darbepoetin alfa (Aranesp; Amgen), whose prolonged duration of action means that it can be administered less often while maintaining a similar safety profile.

The crucial difference between rHuEPO and darbepoetin alfa that prolongs the activity of the latter is the attachment of two extra sialic-acidcontaining *N*-linked carbohydrates to its polypeptide backbone. In a paper published online in Nature Biotechnology, Elliott and colleagues describe the method by which they manipulated the glycosylation pattern of rHuEPO to effect this structural change.

The rHuEPO polypeptide undergoes posttranslational glycosylation at three specific asparagine (Asn) residues and one serine (Ser) residue. These residues are within the consensus sequence Asn–X–Ser/threonine (where X represents any amino acid other than proline), the conserved arrangement of amino acids to which carbohydrates preferentially bind. Hypothesizing that increasing the number of these glycosylation sites would increase the likelihood of carbohydrate attachment, Elliott et al. used in vitro mutagenesis to incorporate extra consensus sequences into the rHuEPO polypeptide.

Sixty-two different rHuEPO analogues were generated, twenty-six of which had additional N-linked carbohydrates attached. These extra carbohydrate chains had variable effects on the in vitro bioactivity of individual analogues activity was reduced in some cases, and did not differ significantly from that of rHuEPO in others. Some analogues had normalized structure and

stability. Two suitable consensus sequences — that resulted in analogues that retained activity and structure — were then combined in an analogue that is used clinically, darbepoetin alfa. Structural modelling showed that the two additional carbohydrates that are attached to darbepoetin alfa are the same as those on rHuEPO, and are located in close proximity to the 'native' carbohydrate chains. As this location is distal to the EPO receptor-binding site, attachment of the extra carbohydrates does not interfere with receptor binding, which accounts for the maintenance of the *in vitro* activity of darbepoetin alfa.

In vivo, a single injection of darbepoetin alfa increased haemoglobin levels in mice to a greater extent and for a longer period than administration of a more than tenfold higher dose of rHuEPO. This prolonged efficacy of darbepoetin alfa — its half-life is up to three times that of rHuEPO — has been confirmed in human clinical trials. One recent study indicates that a dosing interval of more than four weeks can maintain haemoglobin levels as efficiently as rHuEPO, which is typically administered two or three times per week. This success, and further experiments reported by Elliott and colleagues showing that glycosylation analogues of leptin and Mpl ligand also had increased in vivo activity and duration of action, indicates that glycoengineering could be a generally applicable strategy for improving therapeutic proteins.

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