Ion chelators for stroke

D-Pharm (Rehovot, Israel) has announced the successful completion of its Phase I safety assessment for a neuroprotective drug candidate DPb99 to improve the clinical outcome in stroke and traumatic brain injury patients[1]. This adds to earlier animal studies that revealed a significant reduction in infarct size and neurological impairments compared with controls following treatment. DPb99 is a novel lipophilic membrane-activated chelator of divalent metal ions that penetrate the cell membrane and then modulate levels of intracellular calcium at the vicinity of the membrane.

Current stroke therapies

With no neuroprotective drugs currently available, the only drug approved for treating stroke is tissue plasminogen activator (t-PA). If given within 3 h of the onset of symptoms, t-PA is considered a safe and effective treatment for certain types of stroke. 'Only approximately one-third of patients having a stroke are not immediately aware of the symptoms, which can occur during sleep,' says Izchak Angel, D-Pharm's Vice-President of R&D. There is also the time necessary to get the patient to hospital and perform CT or MRI scans to exclude haemorrhage, for which t-PA is contraindicated.'

DPb99 has been shown to be effective in animal models for up to 8 h after the onset of ischaemia. 'This compound offers the possibility to address the current challenge of treating stroke victims who are routinely seen in emergency rooms hours after the initial cerebral insult,' says Angel.

Aims of neuroprotection

Brain ischaemia and hypoxia produced by occlusion of the blood supply interrupts cellular respiration resulting in energy failure and loss of membrane potential. The consequent increase in intracellular calcium levels triggers neurotransmitter release, initiating a cascade of events leading to cell death. Abnormal modulation of intracellular and organelle calcium levels is a signal to the cell to undergo processes of programmed cell death or apoptosis. The release of calcium from intracellular stores in the neurons is a key feature of the apoptotic cascade, and if calcium homeostasis can be restored, the cascade could be halted.

The goals of treatment are to interrupt the ischaemic cascade to prevent further neuronal damage and to restore normal cerebral functions. Studies using MRI and PET scans suggest that ischaemia rapidly produces a core of infarcted or necrotic brain tissue surrounded by hypoxic, but potentially salvageable, cells (penumbra)[2]. 'The major target for a neuroprotective agent is to salvage the penumbra and limit the further spread of the death zone,' says Angel. 'We believe that DPb99 stabilizes the ionic perturbations that are a key factor in cell death.'

Previous attempts to limit the calcium flux into neurons by using calcium channel blockers have not been successful because of their associated cardiovascular effects, as well as their inability to influence the release of calcium from intracellular stores.

D-Pharm developed DPb99 for its chelating capabilities while restricting its action to lipid-containing environments. By modifying the structure of the hydrophilic calcium chelator 1,2-bis-(2-aminophenoxyethane)-*N*,*N*,*N*′,*N*′, -tetraacetic acid (BAPTA) with lipid moieties, its affinity to calcium in water environments such as in plasma or extracellular fluids was substantially reduced to such a level that calcium chelation only occurs in lipid environments. Its action therefore specifically targets the cell membrane where it can regulate calcium flux, leaving plasma calcium unaffected.

Preclinical *in vivo* studies

Animal studies have shown that DPb99 is highly efficacious in reducing the effects of ischaemia. In one animal model, male Mongolian gerbils were subjected to global forebrain ischaemia for 10 min. Two groups were treated at the beginning of reperfusion with boluses of 5 or 10 $\mu g\ kg^{-1}$ DPb99, while a third group was treated after 3 h with 10 $\mu g\ kg^{-1}$. DPb99 attenuated a dose-dependent rise in serum neuron-specific enolase (NSE). As NSE is released from dying neurons into the bloodstream, NSE levels should be proportional to the extent of brain damage. In addition, DPb99 showed a prominent neuroprotective effect quantified by cell counts in the CA-1, CA-2 and CA-3 regions of the hippocampus. The effect was observed even when the drug was given 3 h after the start of reperfusion[1].

In another study, presented at the *International Congress of Pharmacology of Cerebral Ischaemia* (1999, Copenhagen, Denmark), male Sprague-Dawley rats were subjected to permanent or transient (2 h) middle cerebral artery occlusion. DPb99 was administered in 5 or 10 $\mu g\ kg^{-1}$ intravenous boluses, 1, 5 or 6 h after permanent occlusion, and immediately, 3 or 8 h after the start of reperfusion in the transient model. In permanent or transient occlusion, DPb99 reduced mortality by 50% compared with controls and significantly improved neurological scores. In the transient model, the cortical infarct size was reduced by 70–90% and the subcortical infarct size reduced by ≈50% compared with controls. In the permanent model, the infarct volume was reduced by >60% compared with controls. The results indicate that DPb99 is effective even when administered 8 h after the onset of ischaemia and at very low doses.

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Angel believes that DPb99 is effective for long periods after the initial ischaemic event because of its specific mechanism of action. 'The target is the penumbra, a zone that is suffering but not yet dead. Blocking the calcium-induced apoptosis and reducing the immediate consequences of calcium-induced depolarization hinders the propagation of neuronal death and gives us a wider therapeutic window.'

Safety studies

D-Pharm has just finished the two phases of the Phase I safety assessment of DPb99 using 0.03–1.00 mg kg^{-1} in single and repeated doses in young and elderly healthy male volunteers. 'We were particularly happy that we saw no cardiovascular effects on ECGs, nor effects on blood pressure and heart rate. The only major side effects observed were reactions at the sites of injection,' says Angel. A Phase II study is currently being planned to begin shortly in stroke patients.

Future studies

Other studies currently being undertaken include investigations whereby DPb99 not only reduces calcium levels at the vicinity of the cellular membrane but replenishes calcium stores when these are depleted.

D-Pharm is developing its Membrane-Activated Chelator (MAC) platform to create additional compounds that could be used for other chronic diseases. 'Copper, iron and zinc ions are all thought to play important roles in chronic neurodegenerative disorders, such as Parkinson's and Alzheimer's disease. We are currently investigating additional MAC compounds in animal models of these diseases.'

REFERENCES

1 Tolmasov, M. *et al.* (1999) DPb99: a novel membrane-targeted compound with a strong neuroprotective action in cerebral ischemia. *Neurosci. Lett.* 54 (Suppl.), S41

2 Heiss, W.D. (2000) Ischemic penumbra: evidence from functional imaging in man. *J. Cereb. Blood Flow Metab.* 20, 1276–1293

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Dietary supplement to treat asthma

Scientists at Pilot Therapeutics (Winston-Salem, NC, USA) have developed a dietary supplement to inhibit the production of leukotriene B_4 (LTB_4), one of the key mediators of inflammation in asthma. The main constituents of the supplement are the (*n*-6)-series fatty acid γ-linolenic acid (GLA), which is mostly found in the oils of evening primrose and borage seeds, and the (*n*-3)-series eicosapentaenoic acid (EPA), an inhibitor of the enzyme Δ-5-desaturase, which is involved in the production of arachidonic acid from fatty acids such as GLA (Ref. 1).

Leukotrienes such as LTB_4, LTC_4 and LTD_4 are produced from essential fatty acids via the metabolism of arachidonic acid through the lipoxygenase metabolic pathway, and are known to play an important role in the asthmatic response. LTB_4, for example, is released by activated mast cells and macrophages and acts as a chemoattractant to neutrophils and eosinophils, which cause airway inflammation. In theory, as the quantity of LTB_4 produced in the inflammatory cells of people taking GLA is reduced, the fatty acid could be a useful adjunct to inhaled asthma therapy[1]. Moreover, GLA has already been shown to relieve the signs and symptoms of other inflammatory conditions such as rheumatoid arthritis and ulcerative colitis[2].

GLA – the paradox

The ability of GLA to reduce inflammation is paradoxical because it is also a potential metabolic precursor of arachidonic acid, which itself is transformed in the liver into leukotrienes and prostaglandins. Therefore, says Floyd Chilton, Chief Scientific Officer of Pilot Therapeutics, 'Given alone, GLA causes an increase in serum arachidonic acid resulting in an attenuation of its anti-inflammatory activity and, more importantly, potential cardiovascular adverse effects via the formation of platelet-aggregating endoperoxides and thromboxanes.'

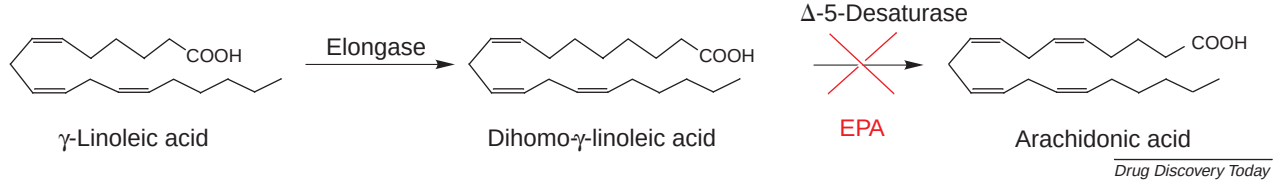

Figure 1. *The Δ-5-desaturase inhibitor, eicosapentaenoic acid (EPA), inhibits the increase in arachidonic acid levels caused by the dietary supplement, γ-linolenic acid (GLA).*

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