Stretching neurons

Smith and colleagues calculated how fast axons could grow and used these parameters to find the limits of neuron growth and the optimum speed of growth for viability. Neurons were plated onto adjacent membranes and allowed to integrate across a 50 μm border between the membranes. The neurons were then progressively stretched by using a microstepper motor system to separate the two membranes at a rate of 3.5 μm per 5 min. The neurons not only stretched to at least 1 cm in length after 10 days, but also formed bundles comprised of thousands of axons, thus generating a nerve fibre structure similar to that found *in vivo* (Fig. 1). This represents an insight into the mechanism of growth of white-matter tracts during development.

Smith says this was an unexpected finding: 'The first thing I had in my mind was that we would have a long single cell, but instead, they kept grouping more and more together, coalescing into larger and larger bundles that you can actually see with the naked eye, about the size of a human hair.' Smith thinks that this is part of what occurs in development: first, you have a group of neurons signaling to each other and as they grow, they cluster together to form bundles. This was an important goal for Smith and colleagues: to accomplish not only length, but number as well because, as with many cell types, it can be speculated that strength in numbers can afford growth advantages to the cells.

Future studies

Smith and colleagues now intend to study the mechanisms of stretch-induced growth and will be testing their stretched-axon technology in animal models to show that these neuron grafts are connecting with the host tissue with a complete connection; this could be demonstrated by flowing a dye across the graft region and showing that an electrical current is able to traverse the graft. The ultimate aim is to develop successful transplant therapies for neurodegenerative diseases such as Parkinson's disease, CNS injury and optic nerve damage, and also to expand their studies to include peripheral nerve repair.

Reference

1 Smith, D.H. *et al.* (2001) A new strategy to produce sustained growth of central nervous system axons: continuous mechanical tension. *Tissue Eng.* 7, 131–139

The end for diabetic kidney disease?

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Preclinical studies suggest that an inhibitor of amadorase, the enzyme at the centre of a newly discovered metabolic pathway that contributes significantly to the development of diabetic nephropathy, could offer treatment hopes to diabetic patients. The compound, DYN12, could help to delay, or even prevent, the onset of serious kidney problems. This approach is one of several currently being developed to tackle the range of life-shortening complications that arise in diabetics. 'Antioxidants and inhibitors of the β isoform of protein kinase C are being considered for cardiovascular and renal complications, and specific anti-angiogenic drugs, such as integrin antagonists, are being developed for proliferative diabetic retinopathy,' says Michael Brownlee (Albert Einstein College of Medicine, Bronx, NY, USA). However, Brownlee is impressed by the potential of DYN12 and considers it to be 'one of the more exciting new strategies'.

What is amadorase?

Amadorase, a previously unknown fructosamine kinase, was discovered, purified and characterized by scientists who later founded Dynamis Therapeutics (Wyndmoor, PA, USA). 'We have shown that amadorase is responsible for the production of 3-deoxyglucosone (3DG), a highly reactive dicarbonyl sugar that is a precursor to the advanced glycation end-products (AGEs),' explains Annette Tobia, President and CEO at Dynamis. AGEs were first shown by Brownlee to form on the surface of proteins, and to enable them to cross-link[1]. These cross-linked proteins cause much of the damage to the glomerular basement membrane that leads to problems in the kidneys of patients with diabetes. Previously, it was thought that 3DG resulted exclusively from non-enzymatic rearrangement, dehydration and fragmentation of a fructoselysine-containing protein (Fig. 1). Tobia and colleagues have now shown that 3DG is also produced as a by-product of a pathway that recovers lysine from fructoselysine (Fig. 1). 'This enzymatically-controlled process is the major source of 3DG in the body,' confirms Tobia.

There is considerable clinical and experimental evidence that 3DG is a major factor in the development of diabetic nephropathy. For example, elevated levels of 3DG and 3-deoxyfructose (its

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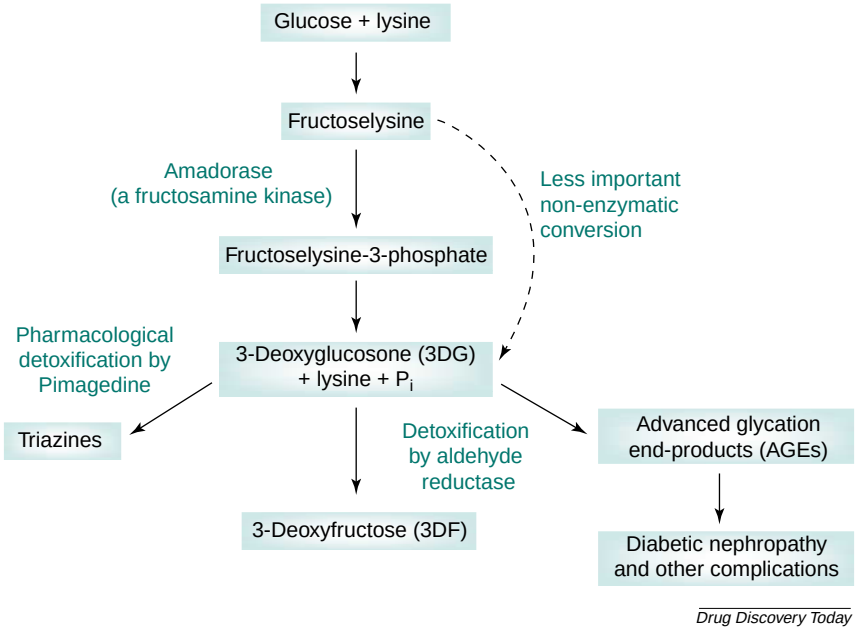

Figure 1. 3-Deoxyglucosone (3DG) pathways. A reversible reaction between glucose and the amino groups of lysine-containing proteins initiates the formation of the stable ketoamine, fructoselysine. Significant amounts of 3DG are produced enzymatically from fructoselysine by amadorase. 3DG is either detoxified or goes on to form harmful AGEs. Pimagedine converts 3DG to harmless triazines.

detoxification product) are found in the plasma and urine of diabetics but not in non-diabetics[2]. 3DG is particularly elevated in patients with signs of diabetic nephropathy. Also, Pimagedine (Alteon; New Jersey, NJ, USA), an inhibitor of AGE cross-linking, has been shown to reduce AGE associated renal pathology in animal models. 'Phase III clinical trials of Pimagedine showed that AGE inhibition led to a significant reduction in progression of diabetic nephropathy, compared with conventional treatment using angiotensin-converting enzyme (ACE) inhibitors,' explains Brownlee. The randomized, double-blind ACTION 1 (A Clinical Trial In Overt Nephropathy) trial involved 690 patients at 56 clinical sites in the USA and demonstrated a 28% reduction in nephropathy, suggesting that Pimagedine also slowed the progression of retinopathy[3].

Preclinical studies

After isolating and purifying amadorase, scientists at Dynamis showed that DYN12, an effective *in vitro* inhibitor of amadorase, could reduce 3DG levels in the plasma of diabetic rats. In the treated group, the rats showed a 53% reduction of 3DG levels compared with a control group that received only saline. 'The ability of DYN12 to reduce systemic 3DG levels suggests that other diabetic complications could also be potentially treatable. We are already starting to look at atherosclerosis and nerve complications but don't currently have the resources to investigate retinopathy,' reports Tobia. 'Phase III trials indicate that blocking the effects of AGEs has a significant effect on diabetic renal complications; because the role of DYN12 will be to prevent the formation of AGE precursors, rather than to block them once they have been produced, the Dynamis approach could have even greater efficacy,' predicts Brownlee. However, the competition from Alteon is hotting up. In January it announced preclinical results for its own AGE-formation inhibitor, ALT946, showing that this drug was more effective than Pimagedine, both *in vitro*, and *in vivo* in the diabetic rat model[4].

Future studies

Both companies are well aware that the clinical impact of an agent that could prevent diabetic nephropathy and could potentially reduce the incidence of other diabetes-associated complications would be enormous. 'Diabetes is the most common cause of renal failure, blindness and lower-limb amputation in the working population – it is also a major risk-factor for heart attacks, with about one-third of all heart attacks occurring in diabetic patients,' says Brownlee. Dynamis is, therefore, urgently searching for a corporate partner to extend its development of DYN12. 'Although preclinical work is continuing, we are not yet able to embark on the two-year toxicology study required before clinical trials could begin. As soon as we form a partnership to fund the next stage of the work, the first stage of clinical investigations could begin within two-and-a-half years,' says Tobia. If this is done successfully, Tobia anticipates that patients will be tested for elevated levels of 3DG as a marker for nephropathological risk. 'Only patients who are likely to develop kidney complications as a result of their diabetes would need to receive treatment, but a truly preventative therapy could benefit millions of people worldwide,' she predicts.

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