METABOLISM provides alternative door to stroke therapy

Even in the age of genomics and gene hunters, a holistic view of cell metabolism can result in revolutionary new drugs.

By Dr Keith F. Martin and Tony Dolan

You know that feeling you get the day after an exaggerated attempt at physical exercise, when you suddenly feel sore muscles in places you didn't know you had any. To the biochemist, this morning after stiffness is textbook material. The muscle ran out of oxygen which the mitochondria – the cell's power stations – need to burn the glucose derived from carbohydrate foodstuff. In this case, the metabolism produces a dead-end product called lactate that is removed so slowly that it still makes you feel bad the next day.

The muscle will have forgotten all about the oxygen crisis three days later, but the brain is much more sensitive to failures of energy supply. Although it makes up only 2% of body weight, the brain of a person at rest consumes around 20% of the oxygen utilised by the body as a whole. Lack of oxygen in the brain can easily become life threatening in a variety of situations including birth complication, decompression of airplanes, heart failure and stroke.

Stroke is the most common and widespread kind of energy crisis in the brain and its effects are as devastating for society as a whole as for the individual. It accounts for more than half of the acute neurological hospitalisations in the USA, and leads to total costs of more than $40 billion. Essentially, the situation at the cellular level is similar to the anoxia in the muscle – the arrest of blood circulation in the vessels blocked by a clot interrupts the oxygen supply. Metabolism produces lactate which by its acidity makes things worse. In most stroke cases, the indirect damage from this loss of blood supply (ischaemia) is far bigger than the direct damage by broken blood vessels surrounding the site of the primary event.

What can be done to stop this damage after a stroke? So far, doctors can do nothing but try to dissolve the blood clot and hope that at least parts of the damaged brain regions will slowly recover. But some hope for new therapies is found in the independent work of Richard Veech (USA) and Shimizu Pharmaceuticals Co Ltd (Japan). After careful analysis of the complex network of reactions that make up the power generator of the mitochondrion, these researchers have come up with a suggestion of an alternative therapy that could help save lives and livelihoods of stroke patients and others. Because feeding glucose to the oxygen-deprived cell would only make things worse (because of the very same lactate accumulation which makes the muscle feel sore), Veech and the Shimizu group independently entered metabolism by a completely different door, the ketone body route, which can supply energy with a much lower oxygen requirement and, surprisingly, decrease cerebral oedema.

This may sound like a heretic suggestion to physicians, as they associate the accumulation of ketones in the blood with the kind of crisis that can kill diabetics. However, researchers studying the physiology of fasting in the 1960s, including Harvard's George Cahill and Eric Newsholme, have shown that a mild enrichment of ketone bodies in the blood is actually useful during starvation and probably the reason why humans are much more resistant to extended periods of fasting than many other mammals. The body's reservoir of storage carbohydrate (glycogen) would only last for a couple of days, and glycerol derived from the triglycerides in fat tissue

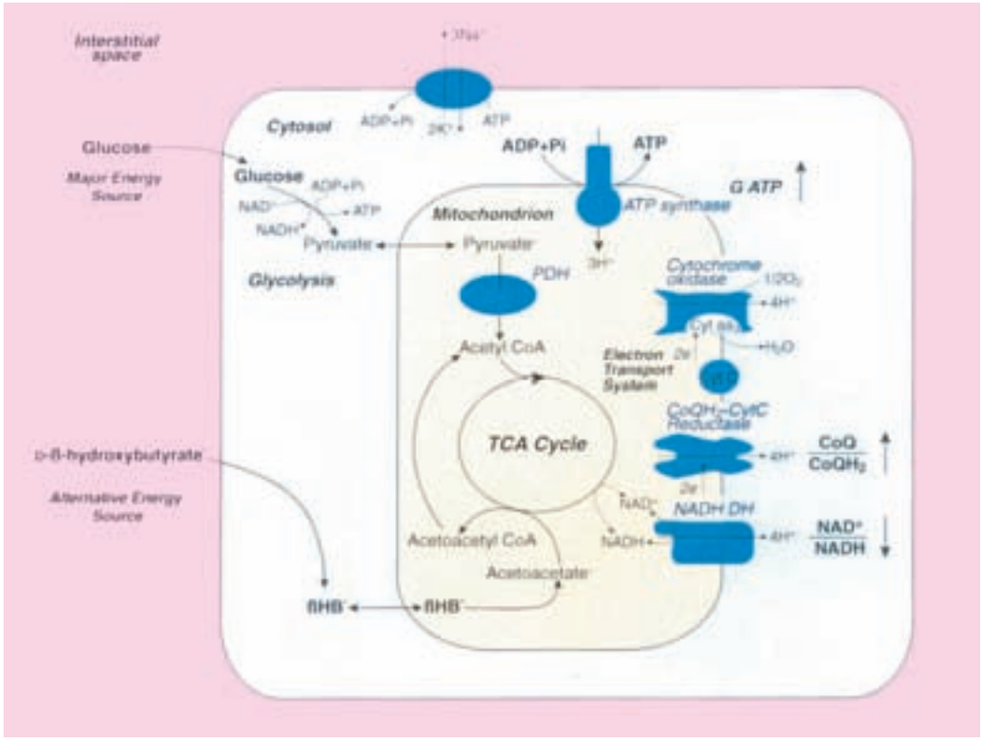

Figure 1

only for weeks. Thus, the brain's ability to use ketone bodies as an energy source increases the survival time of an adult human from two or three weeks to two months. However, with the one notable exception of the so-called 'ketogenic diet' to treat epileptic children, the therapeutic potential of this effect has not been realised.

In the mid-1990s, Veech and his co-workers presented detailed analysis of the metabolic reactions in a rat heart[1,2]. They demonstrated that the control of this complex system is distributed over many components, depending on the overall context. This explains why such networks are surprisingly robust against change. It also suggests that any approaches based on a simplistic molecular genetic idea of targeting one gene or protein (eg an enzyme, receptor or ion channel) may fail to address the complex system in the desired way. In recent years, computer programs have become available which are increasingly able to simulate the metabolic networks of entire cells at least in simple cases such as the erythrocyte[3]. Beyond genomics and proteomics, such holistic views of the natural phenotype are required to come to an understanding of the cell and to an effective approach towards therapies targeted at it.

In the particular case of the mitochondrial energy metabolism, his detailed analysis of the complex regulatory network led Veech to realise that ketone bodies (D-ß-hydroxybutyrate and acetoacetate) can serve as an alternative energy source to glucose. In the rat heart experiments, they even mimicked the effect of the insulin signal (which tells the cell that food is available). Furthermore they can enter the Krebs cycle on a more direct route: as their carbons are already more oxidised than those of glucose, they can skip glycolysis, which means they require less oxygen (**Figure 1**). This finding solved a mystery that had remained unexplained for half a century – why sperms treated with these ketones become more mobile while using less oxygen. In a recent hypothesis paper[4], Cahill and co-authors propose that the supply of ketones to tissues could be therapeutic in a variety of conditions including Alzheimer's and Parkinson's disease. Indeed, Veech and his collaborators at Tattori University (Japan) already demonstrated neuroprotective effects of D-ß-hydroxybutyrate in *in vitro* models of both of these chronic neurodegenerative disorders.

Motohisa Suzuki and co-workers at the Shimizu Research Center in Shizuoka, Japan, conducted experiments to test the effects of ß-hydroxybutyrate by intravenous injection on oxygen-deprived brains[5]. In all of the conditions examined, application of the ketone body considerably improved survival times in comparison with saline controls and

References

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5 Suzuki, M et al (2001). Effect of ß-hydroxybutyrate, a cerebral function improving agent, on cerebral hypoxia, anoxia, and ischaemia in mice and rats. Jpn. J. Pharmacol. 87 143-150.
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also with glycerol, a hypertonic agent that reduces cerebral oedema. Specifically, these workers showed that D-ß-hydroxybutyrate significantly reduced cerebral oedema and infarct size. This finding is particularly exciting because it suggests that in spite of the failure of all of the 29 potential neuroprotectant compounds that have reached clinical trials in stroke and head trauma, there is a real opportunity to develop a drug that could protect the cells on application. Such a therapy would provide significant advantages over all existing stroke treatments because the research so far suggests that ketone bodies could be applied as an immediate treatment in acute stroke and head injury.

Using intellectual property rights of both Veech and Shimizu, the technology commercialisation company BTG has now founded a company called KetoCytonyx, Inc to develop ketone bodies for clinical applications against a number of brain diseases and injuries. The new company is based at West Conshohocken, Pennsylvania. George Cahill is the chair of the scientific advisory board.

Acute treatment of the kind of brain damage (from stroke or head injury) that current methods cannot prevent is the initial focus of the company. As the cell protective effect has already been well demonstrated in animal models, and the ketone bodies are natural components of the human physiology which are known to be harmless at the relatively low concentrations required, it is anticipated that the development will move quickly to the first clinical trials, which will initially serve to optimise a form of administration of the ketone bodies to human patients, and then establish their effects and usefulness.

In the long term, the company will also address chronic diseases, including Alzheimer's and Parkinson's disease. Although the precise mechanisms leading to the pathology of Alzheimer's disease are still under intensive investigation, it is believed that pyruvate dehydrogenase, a key enzyme on the glucose route, is inhibited by the presence of the ß-amyloid peptide, the overproduction of which has been shown to be toxic in cells and is a hallmark of the disease. If this is confirmed, the alternative route via ketone bodies might well be able to protect Alzheimer sufferers from the cell damage and ensuing loss of brain function normally observed in the later stages of this devastating disease.

Unlike the stroke treatment, which would probably be based on injections of a solution containing one of the ketone bodies, chronic treatment could be based on novel oral metabolic precursor formulations designed to supply a constant level of ketone bodies. Such a product could be more user-friendly than either the somewhat unpalatable and potentially atherosclerotic 'ketogenic diet' (lots of fat and no carbohydrate) or repeated injections.

Another major target for the potential use of ketone body therapies in chronic conditions is Parkinson's disease. Although an established treatment for this disease exists (using dopamine precursors or dopamine mimetics), the long-term success of such treatment is somewhat diminished by damage caused by oxygen radicals. Although the effect of ketone bodies in this context has not yet been studied in much detail, it has been suggested that the problems arising from these radicals could be reduced by such a therapy. Similarly, ketone body therapy may be able to prevent oxidation damage in a genetic disease called Friedreich's ataxia, which arises from the failure of a mitochondrial iron export system.

The list of potential applications doesn't even end there, but could be continued to include a number of less frequent diseases and medical problems. It goes to show that, in spite of the current gold rush of the genes, genomes and proteomes, the traditional approach of looking at the phenotype rather than the genotype, ie studying the complex network of reactions happening in the cell, can still pay off. Molecular biology, as exciting as it is, is very much a qualitative and descriptive science at the moment[6]: it describes the genetic make-up of a cell, and tells us whether a gene is active or not. At times, however, exciting insights can still arise from numbers, as they did in the present case from the observation that more energy can be produced with less oxygen consumption. Veech's very ungenomic endeavour to put exact numbers to all those arrows in the biochemistry text books, which he himself describes as 'Quixotic', has laid a sound foundation for a number of new and probably revolutionary therapeutic applications. As the technical possibilities of conducting quantitative analysis in living cells are improving, more research of this kind is needed. DDW

Keith F. Martin, BPharm, PhD, DMS, MRPharmS *is Director of Technology Strategy in the Health, Medical & BioTechnologies Division of BTG. He is a Visiting Professor in Neuropharmacology at the University of Sunderland and a member of the Editorial Board of the British Journal of Pharmacology.*

Tony Dolan, BSc CPA EPA RTMA ROHIM *is a Senior Patent Attorney for BTG. Originally trained as a pharmacologist, he has more than 10 years' experience in creating and developing new ventures based on early stage technologies.*