these would be affected by arsenic. 'In fact, we found that c-myc mRNA and protein levels were reduced in arsenic treated cells, whereas Sp1 mRNA and protein levels remained unchanged. We therefore propose that arsenic acts to inhibit telomerase by reducing the transcription of c-myc, a transcription factor necessary for the production of mRNA from the gene that encodes hTERT, the reverse transcriptase subunit of telomerase,' summarizes Dang.

Future strategies

Dang reports that his group is currently in the midst of sorting out the exact molecular mechanisms of how arsenic inhibits telomerase gene transcription. 'We are studying the effects of reactive oxygen species induced by arsenic to determine how these might mediate both cell death and inhibition of telomerase transcription,' he says.

Trisenox® (arsenic trioxide) injection was approved by the US Food and Drug Administration on 25 September 2000, for the induction of remission and consolidation in patients with relapsed APL, and trials are continuing; a Phase II trial is currently recruiting participants at the Mayo Clinic (Rochester, MN, USA) and others are planned at various centres in the USA. Cell Therapeutics (Seattle, WA, USA), the biopharmaceutical company that manufactures Trisenox®, is currently seeking approval of the drug in Europe. They were granted orphan medicinal product designation in the EU last year and the marketing application is currently under review.

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New stroke therapies – hope for the future

Martina Habeck, Freelance writer

Several new approaches to the treatment of stroke are providing some hope for those who cannot get treatment within three hours of the onset of ischaemia. Stroke is still one of the biggest killers in the western world and the main cause of disability. Despite its clinical importance, treatment options for acute stroke are limited. This has been exacerbated by the fact that many putative treatments have worked in animal models, but few have been shown to work in man.

A stroke occurs when the blood flow to an area of the brain is interrupted; in ischaemic stroke, which accounts for 80% of all stroke cases, this is caused by a blood clot or an occlusion in a blood vessel. The only medical treatment that is available to date, tissue plasminogen activator (tPA), dissolves blood clots if administered within three hours of stroke onset. However, this is a limitation that leaves 98–99% of cases in the USA untreated.

Therefore, there is an urgent need for other therapies that can minimize the damage caused by the interruption of blood flow. Lawrence M. Brass, a stroke expert at Yale University of Medicine (New Haven, CT, USA) and spokesperson for the National Stroke Association (Englewood, CO, USA), says: 'I think people need to begin to think in a broader range of approaches of how to treat stroke, and that is actually what some of the agents [mentioned in this article] did.'

Stopping secondary reactions

The interruption of cerebral blood flow deprives brain cells of oxygen, leading to a reduction in energy production and an associated build-up of toxic metabolites that trigger the ischaemic cascade (Fig. 1). This involves the release of mediators of cell death, including the neurotransmitter glutamate, inflammatory mediators and reactive oxygen species (ROS). Ultimately, these secondary reactions result in brain cell damage and death. Many novel treatment approaches target one of these mechanisms. At the *Annual Meeting of the Society for Neuroscience* in San Diego (CA, USA), 10–15 November 2001, three companies working in this area presented their results (http://sfn.scholarone.com).

Anti-oxidant therapy

Enzymes such as superoxide dismutase cannot cope with the excessive amounts of ROS that are being produced during the ischaemic cascade. Therefore, the free radicals react with cell components and cause damage that leads to neuronal death. To address this problem, Incara Pharmaceutical Corporation (Research Triangle Park, NC, USA) have developed catalytic molecules (metalloporphyrins) with potent anti-oxidant activity. Their lead compound for the treatment of stroke, AEOL1050, significantly reduced the extent of brain damage (infarct volume) and improved

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DDT Vol. 7, No. 3 February 2002

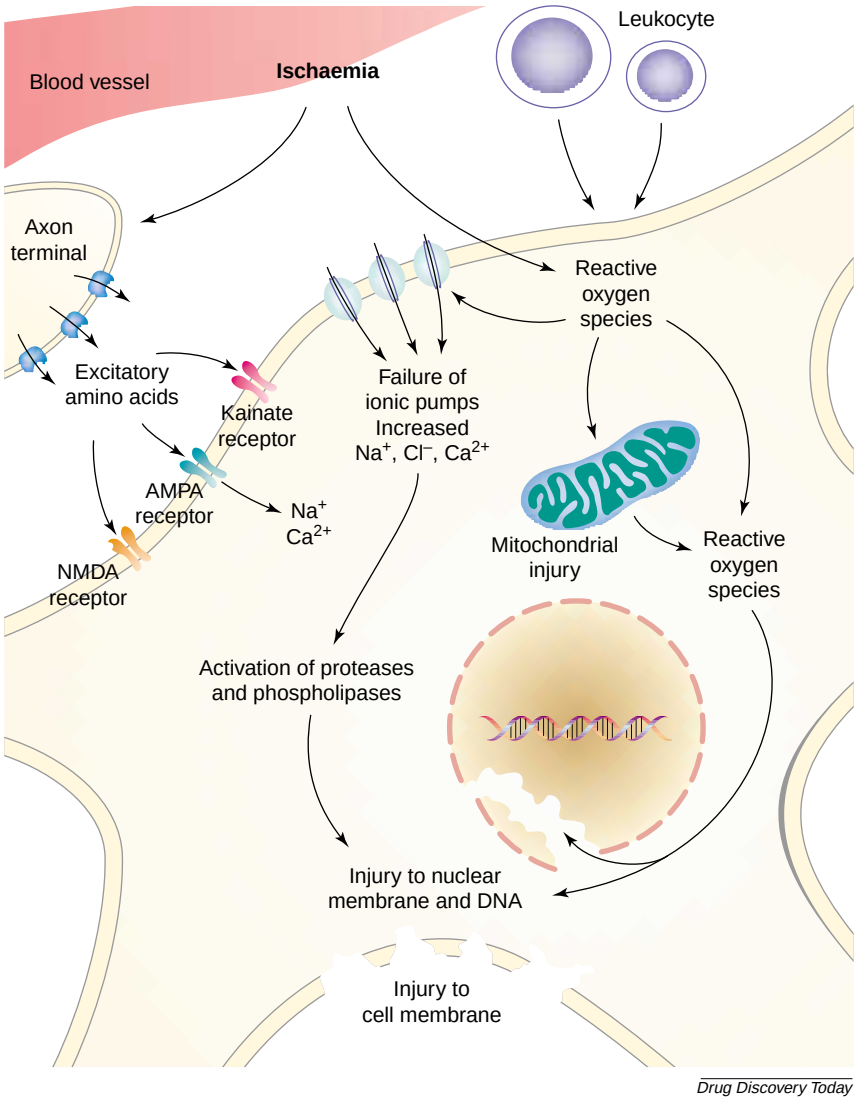

Figure 1. In stroke, the interruption of blood flow triggers a series of biochemical events, collectively called the ischaemic cascade. These events include the release of excitatory amino acids, intracellular overloading with calcium ions, the production of reactive oxygen species (oxidative stress) and local inflammation. The end result is apoptosis. Figure kindly provided by Incara Pharmaceutical Corporation (Research Triangle Park, NC, USA).

function in several stroke animal models, including temporary and permanent middle cerebral artery occlusion (MCAO) models in rats and mice.

Richard Gammans, Senior Vice President of Antioxidant Therapies at Incara, points out, 'We see effects as late as seven hours after the initiation of stroke injury. The implications of this finding are important both in terms of therapy and in terms of the pathobiology of stroke.'

Anti-inflammatory therapy

Researchers at Pharmos Corporation (Iselin, NJ, USA and Rehovot, Israel) are developing non-psychotropic dextrocannabinoids that act as NMDA receptor antagonists with anti-inflammatory properties. George Fink, Vice President of Research, says that the anti-inflammatory action – the ability to inhibit cyclo-oxygenase (COX)-2 activity and chemokine and cytokine production – seems to be the crucial factor when it comes to neuroprotection.

Fink says their lead compound PRS211092 induced 60–70% functional recovery in the so-called staircase test, an objective test to assess skilled paw reaching (for more information see http://www.cf.ac.uk/biosi/staff/dunnett/stairtxt.html), compared with vehicle alone (PEG-ethanol). That correlates with a reduction in infarct volume. The experiments were done in rodents with transient MCAO; the compound was administered intravenously up to three hours after stroke onset. The researchers are currently extending the therapeutic time window to six hours.

Stopping apoptosis

Scientists at Celgene Corporation (San Diego, CA, USA) hope to prevent neuronal apoptosis (programmed cell death) by blocking c-Jun N-terminal kinase (JNK). JNK activity is an important factor in apoptotic neuronal cell death. In a proof-of-concept experiment, the scientists administered the compound SPC9766 to rats, 15 minutes after the onset of transient ischaemia. They found improvements in behaviour and infarct volume. SPC9766 also reduced the number of TdT-mediated dUTP nick end labelling (TUNEL)-positive cells, an indicator of the number of apoptotic cells. Heather Raymon, Project Team Leader of the JNK CNS programme, says, 'This is the first study to show the efficacy of a JNK inhibitor in an animal model of stroke. We are pursuing more potent compounds in further studies.'

Restoring the supply of oxygen and nutrients

In a completely different approach, Neuron Therapeutics (Malvern, PA, USA) has developed a synthetic cerebrospinal fluid, which has been formulated to carry oxygen and carbon dioxide (oxygenated fluorocarbon nutrient emulsion, Revoxyn™). CEO Bruce Shook explains: 'The idea is that there is a route to brain tissue other than the vascular space. If you can utilize this pathway to deliver

oxygen and nutrients to the brain after an ischaemic event, you would basically have a method of stopping the infarction process without having to get things… past a clot that has caused the ischaemic event.'

This idea has proven to be effective in many preclinical experiments performed in cats [1]. According to Shook, the first clinical trial, conducted last year in the USA, confirmed the feasibility of the approach in subjects with very severe ischaemic strokes. In future trials (Phase II), the scientists will look at parameters such as the duration of treatment and the therapeutic time window, which is currently 24 h. If all goes well, the product could be on the market by 2005.

Future potential

According to Brass, all of these approaches show potential but the real test will be whether these compounds are effective in humans. He believes that inhibiting JNK is a promising idea. 'If it works, this would be something that could help people that come in 12 h or a day after the onset of stroke, because programmed cell death does not occur immediately.'

Brass is cautiously enthusiastic when it comes to Revoxyn. 'I think it is a novel idea. I am not sure whether it will work or not but I think it is worth testing in a way that will give us good answers.' He says that part of the challenge is to get the oxygen-carrying fluid inside the brain as well as at the surface of the brain. 'On the other hand,' he concludes, 'a lot of the things we thought would work have not, and it is certainly an innovative approach to try.'

Reference

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A natural antibiotic for cystic fibrosis

Janet Fricker, Freelance writer

A novel antimicrobial peptide, which appears to have a broad spectrum of activity and can overcome drug-resistance problems, has shown promise in Phase I trials for treating patients with cystic fibrosis (CF) [1]. The new compound, iseganan hydrochloride (formerly IB367), which was developed by IntraBiotics Pharmaceuticals (Mountain View, CA, USA), is a synthetic compound based on the natural protegrin peptides found in the human body (Fig. 1).

CF is an autosomal recessive disorder caused by a defective gene on chromosome VII, coding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The commonest mutation results in failure of the CFTR protein to reach the surface of the cell leading to abnormal chloride secretion. In the lungs, impaired chloride transport into airways, with excessive absorption of fluid, produces viscous secretions, which interfere with ciliary transport and facilitate infection and inflammation. The mean predictive survival of patients with CF is 31 years [2].

Current therapies

The goal of antibiotic therapy in CF is to reduce the burden of chronic infectious destruction of lung tissue, improve patient well-being and the ability to live independently, and, ultimately, to prolong survival. Recent advances in CF antibiotic therapy have made it possible for patients to inhale antimicrobial drugs, offering advantages over parenterally administered antibiotics because drugs are delivered directly to the site of infection, higher local doses can be achieved than with systemic use, and the normal flora of the gut and urinary tract are less exposed. The first of these drugs was tobramycin solution for inhalation, which was approved by the Food and Drug Administration in 1997. Unfortunately, continued use of this drug has resulted in the development of antibiotic resistance.

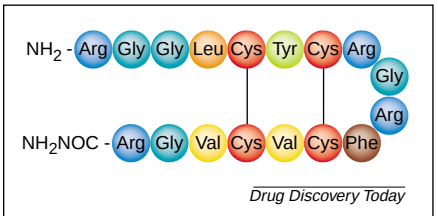

Figure 1. Structure of iseganan HCl. The synthetic antimicrobial peptide consists of a disulfide pleated β-sheet.

Another agent that has been successfully delivered by aerosol is colimycin. Colimycin apparently does not lead to the development of resistance, presumably because of the rapid mechanism of destroying microbial membranes. However, it has a narrow spectrum and can only treat Gram-negative bacteria, leaving Gram-positive bacteria untouched.

Iseganan

'Iseganan represents a significant advance because it possesses the resistance properties of colimycin, with the treatment

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