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Arsenic: carcinogen or cancer therapy?

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A new study has helped to explain why arsenic is an effective treatment for a subtype of leukaemia but is also carcinogenic. The study shows that arsenic is a selective inhibitor of telomerase and this decreased telomerase activity leads to chromosomal end lesions that promote either genomic instability and carcinogenesis, or cell death.

Telomerase and cancer

Telomerase is the enzyme that adds telomere repeats to chromosomes at each round of cell division. This process is vital for chromosome stability; a gradual wearing down of the ends of the chromosomes, which would otherwise happen every time the cell went through mitosis, would delete important genetic information and, therefore, render the cell dysfunctional. Telomerase is, however, also important for continuous cell proliferation in many forms of advanced cancer. 'Since most cancer cells that lack telomerase show sluggish growth and death, telomerase has become an attractive target for anticancer treatment,' explains Chi Van Dang (Johns Hopkins University School of Medicine, Baltimore, MA, USA), senior author of the study [1].

Using arsenic to treat acute promyelocytic leukaemia

Recently, studies have identified arsenic trioxide as a powerful and dramatic therapy for acute promyelocytic leukaemia (APL), a form of adult acute myeloid leukaemia that involves a chromosomal translocation. All-*trans* retinoic acid has proved useful for treating APL in 95% of cases but resistance to retinoic acid therapy occurs in the other 5%; arsenic trioxide is an effective last option treatment for these cases and recent clinical trials have shown that it works well.

Stephen Soignet (Memorial Sloan-Kettering Cancer Center, New York, NY, USA) investigated the safety and efficacy of arsenic trioxide in 40 patients with relapsed APL [2]. Daily infusions of arsenic trioxide were given, for a maximum of 60 doses or until all leukaemic cells in the bone marrow were eliminated. Of the 40 patients, 34 experienced complete remission after the first round of arsenic trioxide therapy. A consolidation course was offered to patients 3–4 weeks later and some patients underwent allogeneic or autologous transplant after arsenic treatment. Eighteen months after treatment, 66% of patients were still alive and 56% were relapse-free [2]. 'The results were outstanding, bearing in mind this form of leukaemia is usually intractable after relapse,' stresses Dang.

How arsenic affects cells from APL patients

Dang and colleagues were investigating the mechanism of action of arsenic in cells derived from APL patients when they made the unexpected link between arsenic and telomerase. They noticed that APL cells that were cultured with arsenic all died in about four weeks; those that survived a three-week exposure to 0.75 μM arsenic showed striking chromosomal end-to-end fusion. From 80 karyotypes, there was prominent polyploidy in 32 cells, and an average of 2.4 fusion events per cell.

Fluorescent *in situ* hybridization (FISH) using a telomere-specific probe demonstrated that these chromosomal fusions were associated with telomere shortening or disappearance. When the telomerase activity of the cultured cells was measured, it was found to be reduced severely after eight days of incubation with arsenic, although chromosomal fusion was not observed to any significant extent until two to three weeks of incubation. However, a control experiment revealed that arsenic could not affect the telomerase enzyme directly. 'We then decided to investigate whether arsenic was acting on one of the genes required to form telomerase,' says Dang.

Discovering the molecular mechanism

Analysis of the mRNA levels in different cell types exposed to arsenic showed that the suppression of telomerase activity correlated with a dramatic decrease in the mRNA for the *hTERT* gene [1], which encodes the reverse transcriptase subunit of human telomerase. Cells treated with two other chemotherapeutic agents, vincristine and doxorubicin, did not show significant changes in hTERT mRNA levels. 'Inhibition of hTERT expression is therefore relatively specific to arsenic, and can be seen in cancer cell lines other than those cultured from patients with APL.

The mechanism was further elucidated by looking at how arsenic inhibits hTERT expression. 'There were two possibilities; either arsenic was inhibiting transcription of hTERT, or it was shortening the half-life of hTERT mRNA,' explains Dang. The half-life of hTERT mRNA is relatively long at four hours, and was not shortened by exposure of cells to arsenic, and nuclear run-on studies revealed that rTERT transcription was indeed severely diminished in arsenic-treated NB4 cells [1]. The *hTERT* promoter contains binding sites for the transcription factors c-myc and Sp1, and so it was likely that one or both of

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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.

References

1 Chou, W-C. *et al.* (2001) Arsenic inhibition of telomerase transcription leads to genetic instability. *J. Clin. Invest.* 108, 1541–1547

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New stroke therapies – hope for the future

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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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