Targeting cancer: a NOvel way

Martina Habeck, freelance writer

A nitric oxide (NO) prodrug has been shown to selectively kill NO-sensitive leukaemia cells, while leaving healthy cells unharmed. The agent, called JS-K, could serve as a promising lead compound for the development of a novel class of anti-cancer drugs.

A remarkable molecule

Few biologically active molecules are as simple and as versatile as NO. The gaseous compound is released by certain immune cells, such as macrophages, to attack microbes and tumour cells. In the brain, it acts as a neurotransmitter, and in the cardiovascular system, it is a vasodilator and helps control the blood pressure. However, depending on the NO concentrations and the cellular surroundings, the free radical can also have deleterious effects by inducing inflammation or DNA damage.

Due to its ability to inhibit tumour growth, NO has become an attractive molecule for cancer therapy. Some scientists are trying to develop gene therapies using vectors that express the NO synthase, and Joseph Bauer at the Cleveland Clinic in Cleveland, Ohio (http://www.clevelandclinic.org) has developed a vitamin B12-based NO generator [1]. None of these therapies has reached clinical trials yet.

There are now reports of a new approach, taken by Paul Shami at the University of Utah (http://www.utah.edu) and Larry Keefer at the National Cancer Institute (NCI; http://www.nci.nih.gov). Shami observed several years ago that leukaemia cells are unusually sensitive to the cytotoxic effects of NO and, ever since then, he has been hooked on research into strategies to use those properties of NO clinically.

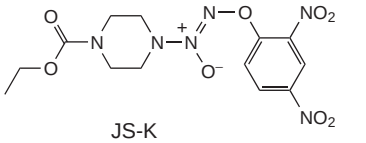
Developing a selective NO donor

However, using NO in the clinic comes with many challenges, because the molecule can have such a wide variety of effects. One of the main problems is its effect on blood pressure. 'If you give just a spontaneous NO generator, several of which are available on the market for blood pressure treatment, you may get significant hypotension without necessarily affecting leukaemia or tumour cell-growth,' warns Shami.

He therefore entered into collaboration with Keefer, who is developing strategies to selectively deliver NO to target sites. One of his approaches involves the design of prodrugs that will only release NO if they are enzymatically activated. He provided Shami with a library of ~50 compounds that target glutathione *S*-transferase (GST), an enzyme that has a key role in detoxification and that is upregulated in a variety of tumour cells, including acute myeloid leukemia (AML) cells. (In that situation, GST has a role in inducing resistance to cancer chemotherapy.)

When Shami and colleagues screened the library compounds for antileukaemic effects, JS-K (Fig. 1) turned out to be the best one *in vitro*. Other experiments in tissue culture confirmed that the agent induced apoptosis in a concentration- and caspase-dependent fashion [2]. The team was concerned about the effects of NO on the vasculature; therefore, they then measured blood pressure in mice treated with intravenous JS-K. 'We were actually able to push the dose to a fairly high level without observing a drop in the blood pressure,' says Shami.

Encouraged by those results, the investigators determined whether JS-K can inhibit the *in vivo* growth of



JS-K

Drug Discovery Today

Figure 1. The structure of JS-K. JS-K is from a series of arylated diazeniumdiolates developed by Joseph Saavedra at the National Cancer Institute (NCI; http://www.nci.nih.gov). Diazeniumdiolates contain the [N(O)NO] functional group, which is a NO-generating moiety. The added aryl ring makes the release of NO dependent on activation by the enzyme glutathione *S*-transferase (GST).

leukaemia and solid tumour cells. Immune-deficient mice were implanted with HL-60 cells – an AML cell line, AML being the most common and most aggressive form of leukaemia – or with PPC-1 cells, a prostate-cancer cell line. In both cases, JS-K inhibited the tumour-cell growth by more than 50% [2]. When Shami and colleagues looked at the tumours histologically, they observed that JS-K had triggered extensive necrosis.

This is extremely good work, comments J. Brice Weinberg at Duke University (http://www.duke.edu), who had been working with Shami before the latter moved to Utah. 'They not only have good *in vitro* data, but have taken big steps beyond that by quantifying NO release in tissue culture and doing *in vivo* experiments in mice.'

Future promise

According to Weinberg, it will now be important to check for other side effects; for example, the investigators might want to measure blood cell counts or study the effect of the drug

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on normal blood cells. If those experiments go well, JS-K could look into a bright future. 'JS-K could possibly be used as a single agent, but I think it would also be a great drug to use in combination with other agents. There is nothing that we have currently available that works through that mechanism,' says Weinberg. Shami is already planning studies to combine JS-K with other standard chemotherapeutic agents to see if they can induce synergy.

The preclinical development of JS-K is also supported by the NCI. It has included the compound in its Rapid Access to Invervention Development (RAID) programme, which is aimed at speeding up the preclinical development of promising drug candidates. 'They have screened JS-K against a panel of 60 different cancer cell lines; it actually has a broader spectrum of activity than what we found it is active in,' hints Shami.

He and Keefer are now also working on optimizing the molecule. 'It is an interesting lead,' says Shami. 'It may not be the end product – we may develop a better compound still. But I think this would be a new class of chemotherapeutic agents.'

References

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Nanodevices hold promise for gene therapy

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The integration of nanotechnology with biology and medicine has been limited thus far to using bioconjugated nanoparticles, such as nanogold or quantum dots, for subcellular detection and tracking of biomolecules or as vessels for delivering drugs, such as antisense oligonucleotides [1–3].

Now, Tatjana Paunesku and Gayle Woloschak at Northwestern University, Chicago (http://www.northwestern.edu) and their colleagues at Argonne National Laboratory (http://www.anl.gov) have developed nanodevices that are able to target, bind and cleave DNA [4]. This novel approach to intracellular manipulation could become the next big thing in gene therapy – or the smallest!

Shedding light on TiO_2

The nanodevice is a chemical–biological hybrid composed of oligonucleotide DNA covalently attached to titanium dioxide (TiO_2) nanoparticles that are only 4.3 nm in size. The nanoparticle exhibits distinctive physical and chemical properties, because of the nature of TiO_2 as a wide-bandgap semiconductor and the increased surface area, compared to bulk TiO_2. 'Corner defects' make the nanoparticle more reactive and enable a stable covalent bond to be formed between it and dopamine end-labeled oligonucleotide DNA.

TiO_2 is also a photocatalyst: when illuminated, the absorption of photons with energy greater than it's bandgap (3.2 eV) results in excitation of electrons from the valence band to the conduction band, generating an electron hole pair, which acts like a positively charged particle. This translates into a unique photoinduced endonuclease activity when these electropositive holes are transferred along the DNA bound to the TiO_2 nanoparticle. According to Paunesku, the cumulative effects of repeated oxidation by these electropositive holes cause a double-strand break of the DNA, cleaving it from the nanoparticle.

Double-stranded breakthrough

'This powerful approach opens up future possibilities in medical biotechnology where different biomolecule-TiO_2 nanocomposites can be used in gene therapy,' says pharmacologist Dusica Maysinger at McGill University (http://www.mcgill.ca). In light of the fact that other biomolecules, such as RNA, peptides and sugars, can also be attached to TiO_2 nanoparticles, the possibilities are endless (see Fig.1). Woloschak, Professor of Radiology, envisions the nanodevices as multifunctional. For example, 'One thing we might want to do is attach one molecule of DNA or a peptide that could target it to a particular cellular site, and another peptide that can carry out an effector function,' she says.

The nanodevice can be used as a simple alternative to current methods of drug delivery, where 'you're just dumping DNA into the bag of the cell and hoping it's going to find its match,'

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