Benzodiazepine library yields lupus target

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By studying a library of benzodiazepine analogs, scientists have found a compound that might be useful against the autoimmune disease systemic lupus erythematosus (SLE). Studies with the compound point to a new molecular target that could be exploited to develop other drugs for the treatment of lupus.

Nobody really knows the etiology of SLE, a potentially life-threatening inflammatory disease, which causes skin rashes, arthritis and kidney inflammation. In the USA, it affects ~1.4 million people, ~90% of them women younger than 45 years old, according to the Lupus Foundation of America (http://www.lupus.org).

Both B cells and T cells contribute to causing SLE; B cells by producing pathogenic antibodies and T cells by generating inflammatory cytokines and stimulating antibody production. 'The problem is, not all antibodies are pathogenic, and there are no good molecular targets right now that are specific to pathogenic autoimmune lymphocytes', comments Gary Glick, professor of biological chemistry at the University of Michigan (http://www.umich.edu). Current treatments for severe SLE, such as cyclophosphamide, suppress the entire immune system. Because they are non-selective in killing immune cells, they have side effects that can be severe and sometimes even fatal.

Researchers have tried a variety of alternatives to block autoimmune responses in SLE. Because many autoreactive antibodies in SLE recognize DNA, a DNA mimic is currently in Phase III trials as a tolerizing agent in patients with kidney disease or inflammation. Monoclonal antibodies against cell-surface receptors to interfere with various signaling pathways that are involved in overstimulating B cells are in testing, although some have caused problems with blood clotting. The mild steroid DHEA was under investigation, because patients with SLE have abnormally low levels of this hormone. However, it did not gain FDA approval (on the basis of studies that tested its ability to reduce disease activity).

Screening benzodiazepines

Looking for a new cytotoxic therapy that would be selective for the immune cells that trigger SLE, Glick's team focussed on the benzodiazepines, after seeing published reports that these drugs can have immunomodulatory properties. Searching within a benzodiazepine library developed by Jonathan Ellman, associate professor of chemistry at the University of California, Berkeley (http://www.berkeley.edu), they found that a few benzodiazepine analogs with no substitution at carbon 3 (which abolishes the anxiolytic effect of the drug) produced a rapid cytotoxic effect that turned out to be a result of apoptosis.

The compound, labeled Bz-423, proved cytotoxic only in mice with a SLE-like disorder. 'It had a remarkable selectivity', says Glick. 'We decided to go on with it simply because it appeared to have an effect on splenocytes, which is where auto-antibodies initially develop.'

The team has learned a great deal about the mechanism of action, which is 'pretty unique', according to Glick. The first details appear in a recent report in the *Journal of Clinical Investigation* [1]. This publication 'represents about 20% of our knowledge', he hints. More recent work apparently confirms the hypothesis, and the team has also looked at the cell-type specificity of the compound *in vitro*.

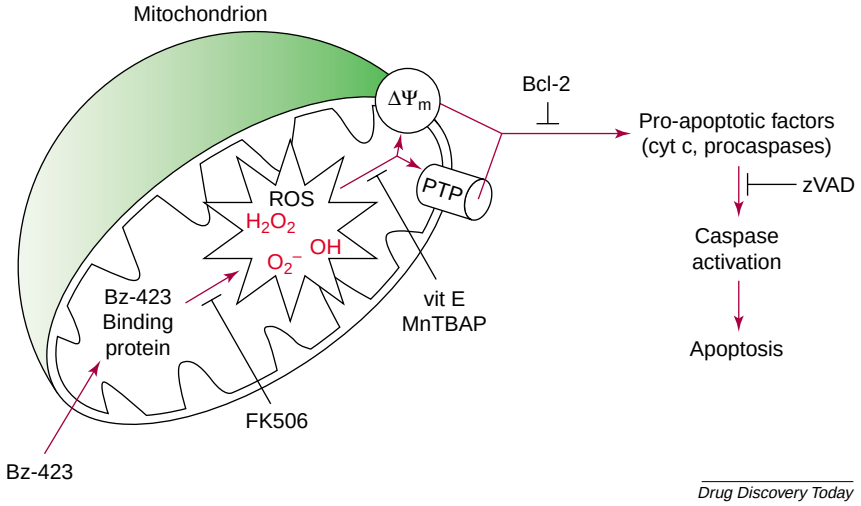

Figure 1. A model of the cellular events induced by Bz-423. Bz-423 binds to a mitochondrial protein generating a FK5-6-inhibitable increase in reactive oxygen species (ROS). The ROS leads to a fall in the mitochondrial gradient ($\Delta\psi_m$) and events consistent with opening of the mitochondrial permeability transition pore (PTP) such as cytochrome c (cyt c) release. These events can be inhibited by antioxidants, such as vitamin E (vit E) and manganese(III)meso-tetrakis(4-benzoic acid)porphyrin (MnTBAP). This process culminates in caspase activation and apoptosis. The general caspase inhibitor *N*-benzoylcarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD) inhibits caspase activation but not cell death. Figure courtesy of Gary Glick, Anthony Boitano, and Katie Johnson (University of Michigan; http://www.umich.edu).

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Experiments using Ramos cells (derived from a human B-cell lymphoma) revealed that Bz-423 localizes to the mitochondria and then very rapidly boosts levels of intracellular superoxide. Rather than being cytotoxic itself, this superoxide acts as a second messenger, a signal triggering apoptosis (Fig. 1).

'This research is going to further the notion that redox signaling is abnormal in lupus', predicts Andras Perl, professor of microbiology and immunology at the State University of New York's Upstate Medical Center (http://www.upstate.edu), who has previously published in this area [2].

To learn more about how Bz-423 promotes B-cell apoptosis, the Michigan researchers pretreated cells with various agents that block known mediators of the process, and watched the effect on the actions of the compound. This revealed the key steps in its cytotoxic effects: cytochrome c release, mitochondrial depolarization and activation of caspase (Fig. 1).

Treatment implications

To explore the action of the agent in live animals, the Glick team chose a mouse model in which disease occurs because of overactive germinal center (GC) B-cells in the spleen, which have some similarity to the Ramos cells used in the *in vitro* studies. GC B-cells produce certain antibodies, which provoke kidney inflammation. Compared with controls, mice treated for 12 weeks with the benzodiazepine analogue had less kidney inflammation (in histology samples), significantly fewer and smaller GC B-cells, and increased apoptotic activity in their spleens.

These results are encouraging, but they do not mean that Bz-423 is going to be as effective in humans. 'This mouse model is largely B-cell-driven, and you cannot really translate findings from the mouse', says Perl. There are several animal models for SLE, and some agents work in one model but not in another.

Whether Bz-423 will eventually become a new drug to treat SLE in humans is anyone's guess. In any case, Glick intends to use it to 'work backwards' and identify some of the cellular defects that cause SLE. He also says that the team will soon publish results using Bz-423 in a T-cell-dominant mouse model. The researchers are also exploring the effectiveness of the compound in various animal models of cancer.

References

1 Blatt, N.B. *et al.* (2002) Benzodiazepine-induced superoxide signals B cell apoptosis: mechanistic insight and potential therapeutic utility. *J. Clin. Invest.* 110, 1123–1132

2 Gergely, P. *et al.* (2002) Mitochondrial hyperpolarization and ATP depletion in patients with systemic lupus erythematosus. *Arthritis Rheum.* 46, 175–190

Neural stem cells as novel drug delivery agents

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Neural stem cells engineered to express interleukin (IL) 12, a tumoricidal cytokine, have been shown, in mice, to track and kill gliomas as they spread through brain tissue [1]. Gliomas are currently treated by using surgical resection with adjuvant radio- or chemotherapy, which can eradicate the main tumour. However, gliomas are highly malignant and remaining cells spread quickly, setting up new tumor satellites. These are extremely difficult to destroy, even using stereotactic radiotherapy, and tumor recurrence is frequent and is associated with poor prognosis.

A stem-cell delivery system?

John Yu and colleagues at the Neurosurgical Institute of the Cedars-Sinai Medical Center (http://www.cedars-sinai.edu/mdnsi/) previously showed that gene transfer of the gene encoding IL-12 into mouse intracranial tumors using adenoviral vectors confers long-lasting immunity and a cytotoxic T-cell response [1]. The team used neural stem cells as a delivery system, hypothesizing that IL-12 secretion in the region of tumor satellites might induce a T-cell response more specifically against these problematic regions of tumor growth. 'It appears from our data that this may be the case', comments Yu.

Evan Snyder's group at Harvard Medical School (http://www.hms.harvard.edu/) first showed, more than two years ago, that neural stem cells can home in and/or track pathology in the adult mouse brain [2]. 'In our study, an oncolytic gene was expressed by the stem cells', explains Snyder, who goes on to say that his group now has a patent nearly issued for this novel approach to treating cancer. 'It is gratifying to see many people beginning to use this technique and validate our findings', he

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