New treatments for cancer and heart disease on the horizon?

Many new candidate treatments for conditions as diverse as cancer and heart disease are likely to enter clinical trials within the next decade, following the recent elucidation of the roles of certain enzymes that are involved in whether cells live or die. These enzymes include C-Jun-*N*-terminal kinases (JNKs), which are signal transduction enzymes that turn certain genes 'on' and 'off' in response to signals from the extracellular environment. Among their targets are the activator protein 1 (AP1)-regulated genes, which are involved in cell division, cell proliferation and cell death[1]. The JNK family includes JNK1, JNK2 and JNK3 and is part of a larger family of kinases, the mitogen-activated protein (MAP) kinases.

Function of JNK1 and JNK2

In the past few months, several papers[2–4] have focussed on the function of JNK1 and JNK2 through the use of antisense oligonucleotides. Two of these studies[2,3] have shown that apoptosis (programmed cell death) usually occurs when JNK1 is activated, while the third study[4] has shown that the growth of human tumour cells is suppressed when JNK2 is inhibited. Antisense technology enables the specific inhibition of gene activity using an oligonucleotide that selectively binds to the mRNA, thus preventing the production of the protein it encodes.

Brett Monia, Executive Director of Molecular and Cellular Pharmacology at Isis Pharmaceuticals (Carlsbad, CA, USA), which has collaborated in all three studies, said, 'Many large pharmaceutical companies have been attempting to identify small molecules to inhibit these proteins for several years, but they have had little success because of the lack of specificity of these inhibitors. Protein kinases are highly conserved at the protein level, so an inhibitor of one kinase often knocks out several and is likely to be toxic as a result. With the antisense approach, we target not the protein but the RNA and we can therefore be more selective. In fact, we can not only discriminate between different classes of protein kinases but can also discriminate between different protein kinase isoforms.'

JNK1 function

In the first of the three studies[2], researchers at Vanderbilt University (Nashville, TN, USA) and from Isis Pharmaceuticals showed that, as expected, erythroid progenitor cells underwent apoptosis when the growth factor erythropoietin (EPO) was removed. There was simultaneous activation of JNK. When expression of JNK1 was suppressed by adding the appropriate antisense oligonucleotide, the proportion of cells undergoing apoptosis was reduced.

A team from Novartis Institute for Biomedical Research (Summit, NJ, USA) and from Isis Pharmaceuticals then reported a rapid activation of JNK during apoptosis in human kidney cells caused by anoxia followed by reoxygenation[3], which is similar to what might occur during organ transplantation. Using JNK isoform-selective antisense inhibitors, JNK1 was found to be responsible for mediating the death of these cells, as inhibition of JNK1 prevented cell death. Hence, active inhibitors of JNK1 might prevent cellular death in reperfusion injury, for example during organ transplantation.

JNK2 function

A collaboration between researchers at the National Institute on Aging (Baltimore, MD, USA) and from Isis Pharmaceuticals investigated the role of JNKs in regulating tumour cell growth[4]. They found that cell growth was significantly reduced on inhibition of JNK2 in cells that had mutations in the p53 gene (and were therefore malignant). Adding antisense oligonucleotides that specifically inhibited JNK2 also caused significant apoptosis in these cultures.

Monia concluded, 'Taken together, our findings strongly suggest that JNK1 is a promoter of cell death, and JNK2 is a protector against cell death. These studies show that these two closely related proteins in the signal transduction pathway can have opposite functions with respect to a common biological endpoint such as cell death.'

The improved understanding of the roles of JNK1 and JNK2 could be therapeutically useful for diseases where unwanted cell death is a problem, such as stroke or myocardial infarction, or where inappropriate cell growth occurs after apoptosis fails to occur, such as cancer.

Future studies

Isis currently has studies underway examining the effects of antisense oligonucleotides that inhibit JNK2 on animal

1359-6446/00/$ – see front matter PII: S1359-6446(00)01519-1

models of prostate cancer, breast cancer and non-small cell lung cancer. 'We have already seen anti-tumour responses in prostate models', Monia said.

Preclinical studies have also begun to examine the effects of antisense oligonucleotides that inhibit JNK1 in mouse models of organ transplantation. 'If we could prevent reperfusion injury from happening in humans having organ transplants, we could increase the chances of the organ being accepted', Monia said.

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Reversing age-related and diabetic cardiovascular disease

Gradual loss of elasticity in the cardiovascular system is an important feature of the ageing process and plays a major role in diseases such as atherosclerosis, hypertension, stroke and heart failure. One of the major causes is thought to be the reaction of glucose with the amino groups of proteins such as collagen and elastin to form advanced glycosylation end-products (AGEs). Over many years, AGEs interact with adjacent proteins to form stable, covalent crosslinks that reduce tissue elasticity. One piece of evidence for this theory is that the stiffening process is accelerated in diabetics. It is also known that agents that inhibit AGE formation (e.g. Pimagedine) can prevent cardiovascular stiffening[1].

Reversing AGE crosslinks

AGE crosslinks were previously thought to be irreversible after their formation. However, researchers at Alteon (Ramsey, NJ, USA) are working on a new class of therapeutic agent that can reverse the crosslinking process and restore the cardiovascular system to a more 'youthful' state. Their lead compound is ALT711 [4,5-dimethyl-3-(2-oxo-2-phenylethyl)-thiazolium chloride], which interacts with the crosslinked proteins, separating them by cleaving the crosslink[2] (Fig. 1). In ageing dogs, ALT711 reversed the age-related increase in myocardial muscle stiffness[3].

Eight dogs with a mean age of 10.6 ± 0.7 years were administered a single oral daily dose of 1 mg kg^{-1} of ALT711 for four weeks[3]. Each dog underwent a baseline haemodynamic study before treatment, and the same evaluation was performed on an untreated control group of seven dogs of similar age. A range of parameters was assessed using echocardiography and invasive catheters linked to pressure transducers. Myocardial stiffness was calculated by an established formula[4], and the assessment was repeated after treatment. The control dogs were also reassessed after four weeks. Treated dogs showed a reduction in myocardial stiffness of ≈40%, accompanied by an improvement in cardiac function as measured by left ventricular end diastolic volume, stroke volume and decreased end diastolic pressure[3]. ALT711 also reduced arterial stiffness in ageing primates and, in this study, the improvement in cardiac output and arterial compliance persisted for up to five weeks after the last dose[5].

In addition to restoring the elasticity of stiffened tissues, ALT711 reversed the pathological hypertrophy of the aorta and left ventricle in animal models of hypertension, with a corresponding decrease in tissue collagen content (E. Frohlich, Alton Ochsner Medical Foundation, New Orleans, LA, USA; unpublished results).

In a related study, conducted by Mark Cooper (University of Melbourne, Victoria, Australia), ALT711 reversed the overexpression of genes for proteins and growth factors known to be associated with pathological hypertrophy (M. Cooper, unpublished data). *In situ* hybridization experiments on kidneys from diabetic animal models demonstrated a reversal of overexpression for Type IV collagen and for the growth factor, TGFβ. These results indicate that restoration of normal tissue dynamics through breaking AGE crosslinks could restore normal control of gene function. Jack Egan of Alteon says, 'In the absence of evidence for a direct effect of ALT711 on transcriptional control mechanisms, it would appear reasonable that [relieving the] stresses on cells and tissues that result in hypertrophy would… lead to normal control of gene activity. Taken

 1359-6446/00/$ – see front matter PII: S1359-6446(00)01523-3