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.

REFERENCES

1 Karin, M. (1995) The regulation of AP-1 activity by mitogen-activated protein kinases. *J. Biol. Chem.* 270, 16483–16486

2 Shan, R. *et al.* (1999) Distinct roles of JNKs/p38 MAP kinase and ERKs in apoptosis and survival of HCD-57 cells induced by withdrawal or addition of erythropoietin. *Blood* 94, 4067–4076

3 Garay, M. *et al.* (2000) Inhibition of hypoxia/reoxygenation-induced apoptosis by an antisense oligonucleotide targeted to JNK1 in human kidney cells. *Biochem. Pharmacol.* 59, 1033–1043

4 Potapova, O. *et al.* (2000) Inhibition of c-Jun *N*-terminal kinase 2 expression suppresses growth and induces apoptosis of human tumor cells in a p53 dependent manner. *Mol. Cell. Biol.* 20, 1713–1722

Sharon Kingman

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

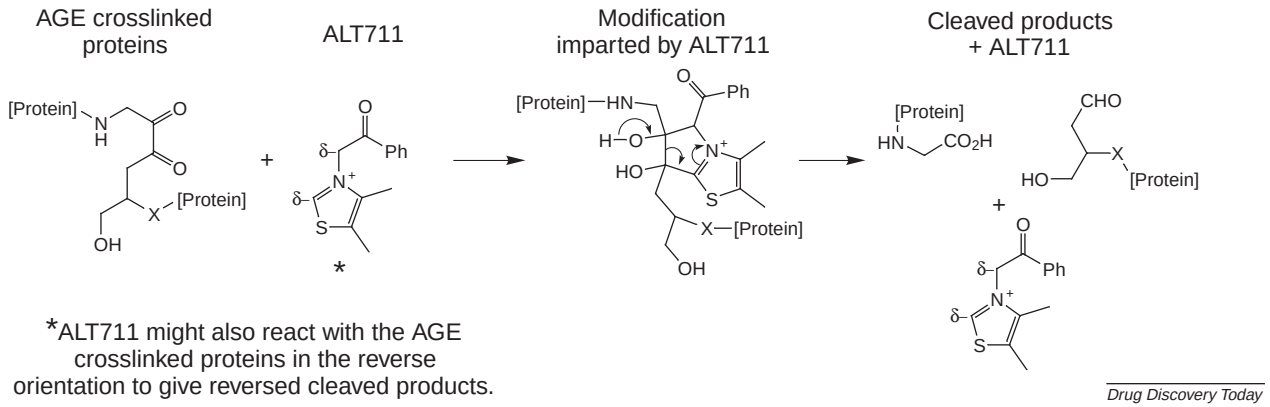

Figure 1. *Mechanism of advanced glycosylation end-products (AGE) crosslink breaking by ALT711.*

together, the rapid restoration of elasticity to stiffened tissues and the resulting correction in gene activity might provide for broad clinical benefit to the stressed cardiovascular system.'

Clinical studies

Phase I clinical studies with ALT711 have not shown any side effects, and it does not appear to disrupt natural enzymatic glycosylation sites or the peptide bonds that maintain the integrity of the collagen chain[2]. A Phase IIa trial was launched in April 2000 and is expected to be complete by the end of the year. This double-blind, placebo-controlled study will involve 72 male and female subjects aged >50 years who have measurable stiffening of the cardiovascular system caused by age and/or diabetes. Their cardiovascular compliance (elasticity) will be monitored using various parameters including ultrasound cardiography and pulse wave velocity. The data will be used to determine which cardiovascular indications will be pursued for further clinical development. These might include congestive heart failure, peripheral vascular disease and isolated systolic hypertension of the elderly.

Alteon believes that AGE crosslink breakers could also be beneficial for many other conditions, such as nephropathy, retinopathy, neuropathy and urinary elastic dysfunction. 'We look forward to additional clinical trials with ALT711 to evaluate whether the stiffening of tissues such as the bladder, peritoneal membrane, muscles in the eye and the elastic joints is similarly caused by this pathological AGE crosslink', says Egan. 'If it is involved in other tissue pathologies, the potential exists for a very broad market not only for ALT711 but for future generations of AGE crosslink breakers currently in preclinical development.'

REFERENCES

1 Brownlee, M. *et al.* (1986) Aminoguandine prevents diabetes-induced arterial wall protein cross-linking. *Science* 232, 1629

2 Vasan, S. *et al.* (1996) An agent cleaving glucose-derived protein crosslinks *in vitro* and *in vivo*. *Nature* 382, 275–278

3 Asif, M. *et al.* (2000) An advanced glycation endproduct cross-link breaker can reverse age-related increases in myocardial stiffness. *Proc. Natl. Acad. Sci. U. S. A.* 97, 2809–2813

4 Douglas, P. and Tallant, B. (1991) Hypertrophy, fibrosis and diastolic dysfunction in early canine experimental hypertension. *J. Am. Coll. Cardiol.* 17, 530–536

5 Vaitkevicius, P.V. *et al.* (1998) Reduction of arterial stiffness in old primates by a novel compound which disrupts vascular collagen cross-links. *Circulation* 98, 1–8

Jo Whelan