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Antisense inhibitor provides new treatment approach for hypercholesterolaemia

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An antisense inhibitor of a protein carrier of low density lipoprotein (LDL) cholesterol has been shown to reduce cholesterol levels in two different mouse models of hypercholesterolaemia, according to scientists from Isis Pharmaceuticals (http://www.isip.com) who presented their findings at the *11th International Congress on Cardiovascular Pharmacology* [1]. Using antisense oligonucleotides as drugs to treat cardiovascular disorders is a relatively new but fast moving area and Frank Bennett, Vice President of Antisense Research at Isis says that, 'If further development is successful, the new antisense drug could complement the widely used statin-based therapies.'

Homing in on an antisense target

Developing antisense drugs depends very much on identifying an appropriate target, and scientists at Isis began by searching the literature to identify components of lipoprotein metabolism produced in the liver. 'The liver is one of the key organs that concentrates oligonucleotides; delivery to the site of production of a target is of primary importance, and exploiting the natural pharmacokinetics of antisense technology seemed sensible,' explains Bennett.

Approximately 30 potential targets are being investigated, including the ApoB-100 protein, which is the major protein component of the LDL-cholesterol transporting molecule (Fig. 1). This protein is expressed in the liver, and it then travels to cells where it acts as a carrier for cholesterol and other lipid. Heterozygous genetically modified ApoB-100 knockout mice have lower cholesterol and appear to be protected from developing diet-induced hypercholesterolemia. 'This mutation is not life-threatening – quite the opposite – so blocking it using antisense technology is not likely to affect normal body functions,' says Bennett.

Antisense provides new strategy against ApoB-100

Traditional drug development strategies have not been used to target ApoB-100 because, unlike enzymes, carrier molecules are difficult to inhibit. The molecules also gain some protection by virtue of their residence in lipoprotein particles. 'Antisense technology, by contrast, is a successful approach because the oligonuceotides that match the sequence of the gene stop translation of the mRNA in the liver; they do not need to gain access to lipoprotein particles,' says Bennett. When the mouse-specific antisense oligonucleotide, currently designated ISIS147764, binds to the mRNA encoding ApoB-100 protein, it recruits RNase H, an enzyme that degrades the mRNA, preventing any translation.

More thorough toxological studies are still to be carried out in the next phase of development, but preliminary studies show that ISIS147764 is taken up into most tissues, and, as expected, accumulates in the liver. 'We have now tested other antisense products in clinical trials in almost 3000 patients, so we are not anticipating any particular tolerance problems with the corresponding human antisense molecule to ApoB-100,' notes Bennett.

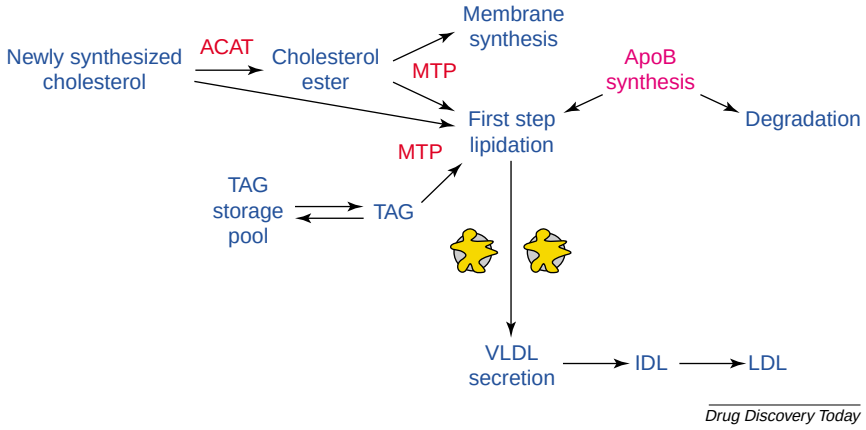

Figure 1. The process of low density lipoprotein biosynthesis. ApoB-100 is the core protein in LDL particles. By inhibiting ApoB-100 through the application of antisense technology using ISIS147764, the production of LDLs is reduced or blocked. Abbreviations: ACAT, Acyl-CoA cholesterol acyltransferase; IDL, intermediate density lipoprotein; MTP, microsomal transferase protein; TAG, triacylglycerol.

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Promising mouse model results

Efficacy is the primary objective of current studies; experiments in mice using the high-fat diet model of hypercholesterolaemia and the genetically modified ApoE knockout model have shown promising results. According to Bennett, in both models, mice that were obese and that had significantly elevated cholesterol levels showed a reduction in overall cholesterol and LDL cholesterol after 3–4 weeks of treatment with ISIS147764. 'The response was dose-related, and was accompanied by a more modest decrease in the plasma levels of trigycerides,' he reports.

Statin-based drugs work more quickly, but ISIS147764 could prove suitable for subgroups of patients who are unresponsive to statins, or for whom statin therapy is contra-indicated. 'From a clinical standpoint, the initial results with ISIS147764 are very exciting,' comments Richard Honkanen (University of South Alabama College of Medicine; http://southmed.usouthal.edu), whose group is also investigating the potential of antisense technology in cardiovascular disease [2]. He points out that ISIS147764 represents the first drug designed to directly treat patients that overproduce ApoB-100. 'Statin therapy, which targets HMG-CoA reductase and lowers LDL primarily by enhancing LDL clearance, is clearly beneficial, but the cardiovascular event reduction is limited to ~30%,' he explains. Because ISIS147764 attacks the problem from the other end – preventing the formation of LDLs via inhibiting the expression of ApoB-100 – Honakanen believes that antisense strategies could bring in a new era for the medical management of heart disease. Bennett agrees, adding that a recent publication suggesting that many more people might benefit from cholesterol-lowering therapy than previously thought [2] adds new impetus to developing antisense drugs that complement statin therapies.

Improving antisense product formulation

The next phase of development will test the new drug in another genetically modified mouse model, and also in a rabbit model of hypercholesterolaemia. 'Antisense therapies are species specific, but the time to identify a rabbit or human ApoB-100 antisense molecule is likely to be less than a month,' confirms Bennett. Several other antisense products are already in clinical trials, and the technology is such that refinements of earlier products will aid the development of those still at the preclinical stage, speeding up this process considerably. 'First-generation products are given by intravenous injection, and are shorter acting, whereas second-generation products have a longer duration of action and can be given subcutaneously, making it possible for patients to give themselves the injections,' says Bennett. The short-term goal is for a preparation that needs to be injected only once every two weeks, but in the longer-term, Isis is also working towards an oral formulation.

Looking ahead to clinical trials

'Our detailed development strategy for ISIS147764 has not yet been worked out; we hope that the next phase will show equally promising results and we will work towards Phase I clinical trials as soon as possible,' confirms Bennett. Although the preclinical development of antisense drugs is much quicker than candidates identified using other methods, the timing from the start of Phase I trials will be more comparable, taking about four years to progress to Phase III trials. 'Phase III trials for an antisense product for hypercholesterolaemia would require a large number of patients, in multiple centres, and we will probably try and set up a partnership with another company to make that possible,' says Bennett.

Reference

1 Bennett, F. (2002) Inhibition of murine APOB B100 by antisense oligonucleotides in a murine model of hyperlipidaemia. *Cardiovasc. Drugs Ther.* 16 (Suppl.) (in press)
2 Golden, T. *et al.* (2002) Use of antisense oligonucleotides: advantages, controls, and cardiovascular tissue. *Microcirculation* 9, 51–64
3 Heart Protection Study Collaborative Group (2002) MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 360, 7–22

Gene therapy progress for HIV

Julie Clayton, freelance writer

The HIV virus could assist in its own downfall if two new gene therapy strategies succeed. Both approaches are due to enter Phase I safety trials in late 2002, using new vectors and protocols that their proponents hope will overcome many of the obstacles of the past 10 years. Researchers led by Carl June, a molecular immunologist at the Abramson Family Cancer Research Institute (http://www.abramsoninstitute.org), reported their progress at the *7th European*

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