illness. 'The organisms you carry on your mucous membranes are the cause of infection 80% of the time, but people don't realize this,' he says. 'We have tolerated these organisms until they cause infection, and then we treat them. Finally we have something to eliminate this reservoir.' Resistance should not be a problem either; Fischetti says they have not seen any resistance at all despite repeated attempts to select for resistant bacteria. Moreover, unlike antibiotics, the lysins only target their specific host and don't affect neighbouring bacteria, thus avoiding disrupting the normal flora. These enzymes are highly stable and could be used in a liquid form as a nasal spray or lyophilized before use. The group is currently working on enzymes from other organisms, including staphylococci, enterococci, and *Bacillus anthracis*. Fischetti says they are currently in discussions with pharmaceutical companies and the food industry to begin Phase I clinical trials with the streptococcal enzymes.

'Both approaches are highly promising,' says Ry Young, an expert in phage biology at the Dept of Biochemistry and Biophysics, Texas A&M University (College Station, TX, USA). 'All bacterial control on earth is done by bacteriophage. There has been a war for billions of years where phage are preying on bacteria,' he says. 'It is clear that there will be many potential applications of phage biology. We are running out of antibiotics, so we have to look at the alternatives.'

References

1 Bermudez, L.E. *et al.* (2001) Killing of *Mycobacterium avium* (MAC) and *Mycobacterium tuberculosis* (MTb) by a mycobacteriophage delivered by avirulent mycobacteria: a model for phage therapy of intracellular bacterial pathogens. *41st Interscience Conference on Antimicrobial Agents and Chemotherapy*, 16–19 December 2001, Chicago, IL, USA (Abstract 1373)

2 Sulakvelidze, A. *et al.* (2001) Bacteriophage therapy. *Antimicrob. Agents Chemother.* 45, 649–659

3 Fischetti, V.A. (2001) Phage antibacterials make a comeback. *Nat. Biotechnol.* 19, 734–735

4 Nelson, D. *et al.* (2001) Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. *Proc. Natl. Acad. Sci. U. S. A.* 98, 4107–4112

5 Loeffler, J.M. *et al.* (2001) Rapid killing of *Streptococcus pneumoniae* with a bacteriophage cell wall hydrolase. *Science* 294, 2170–2172

A 'C' change for hepatitis treatment

Joanne Clough, joanne.clough@drugdiscoverytoday.com

A novel oral compound for the successful treatment of the hepatitis C virus (HCV) has been selected for development by Lilly (Indianapolis, IN, USA) and Vertex Pharmaceuticals (Cambridge, MA, USA). The compound, LY570310 (VX950), is an inhibitor of the hepatitis C protease, NS3-4A, which is considered to be essential for the replication of the virus. LY570310 is the first candidate of a novel class of antiviral drugs that are under investigation for the inhibition of HCV. It is currently in preclinical trials and is expected to enter Phase I clinical trials in early 2003.

The virus

HCV infects 3–4 million people in the USA (according to the Center for Disease Control and Prevention, Atlanta, GA, USA) and the worldwide figure is close to 170 million (according to the World Health Organization; http://www.who.int). Many victims are unaware of the infection, which can remain undetected for up to 20 years; this indicates that worldwide infection could be much higher than estimated. HCV causes inflammation of the liver, which can lead to fibrosis, cirrhosis, liver cancer and, ultimately, liver failure. Complications resulting from HCV infection claim 8000–10,000 lives, annually, in the USA (http://www.hepatitiscaware.org/).

HCV is a small (40–60 nm in diameter), enveloped single-stranded RNA virus of the family *Flaviviridae*. Detection of the disease is through elevated levels of the enzyme alanine aminotransferase (ALT); chronic HCV can result in an increase in ALT levels by up to 20-fold. The disease is initially characterized by flu-like symptoms: aching limbs, fever, headaches, appetite suppression and weight loss. Chronic infection occurs in up to 85% of cases and can be transmitted through the sharing of IV needles (in ~70% of cases) and blood transfusions (before routine testing became available in 1992).

Current treatments

At present, the only treatment for HCV that is approved by the Food and Drug Administration is interferon-α (IFN-α), taken in combination with the antiviral, nucleoside analogue ribavirin [1]. Ribavirin is only approved as a treatment of HCV when used in conjunction with IFN-α (Rebetron™, ICN Pharmaceuticals, Costa Mesa, CA, USA). IFN-α is known to bind to a membrane receptor, which elicits a signalling cascade resulting in the expression of target cell killing by lymphocytes.

Current treatments have only a 40–60% success rate, and can produce unpleasant side effects such as insomnia, depression, extreme fatigue, skin rashes, fevers, nausea and weight loss, which are similar to the symptoms of HCV.

1359-6446/02/$ – see front matter PII:S1359-6446(02)02219-5

Inhibition of viral assembly

The HCV protease, NS3-4A, was targeted for inhibition because of its role in viral replication, where it processes the functional domain (the non-structural proteins) of the genome. Previous targets for the potential treatment focussed on membrane receptors, which trigger virus replication in infected cells. John Thomson, Vice President of Research for Vertex, commented that it had been difficult to find suitable targets to inhibit viral replication because of the flat active site of the enzyme, especially when compared with the binding-site of HIV, for which protease inhibitors have become a new mechanism by which to attack the virus (Fig. 1). There are also the conventional problems associated with virally replicating HCV in the laboratory.

Initial efforts were complicated by the lack of potential interactions between the active site and inhibitors: from the crystal structure of HCV protease [2], structure-based computational and combinatorial chemistry techniques were used to create novel chemical scaffolds. *In vitro* assays were developed by scientists at Lilly and Vertex, which placed the HCV protease domain intracellularly and the subsequent inhibition of viral replication by compound LY570310 was measured. The toxicological profile of LY570310 has been measured in preclinical animal models in mice, rats and dogs. Results from these trials indicate that LY570310 shows good potency, bioavailabilty and pKa values. The efficacy of the compound could prove more difficult to assess because there is no well established animal model and the only robust growth of the virus is in chimpanzees, whose use has several limitations.

President of Vertex, Vicki Sato, commented that, 'Therapeutics that directly inhibit viral assembly paved the way for important treatment advances for patients infected with HIV. While our HCV protease inhibitor has not yet been tested in patients, we are optimistic that drugs such as LY570310 could usher in a similarly significant treatment advance for patients with HCV.' Thompson explained that the significant advances experienced with HIV protease inhibitors was a turning point for the treatment of HCV and provided a 'powerful point of intervention [for HCV inhibition] and is the first time that a target has been aimed at the Achilles heel of HCV'.

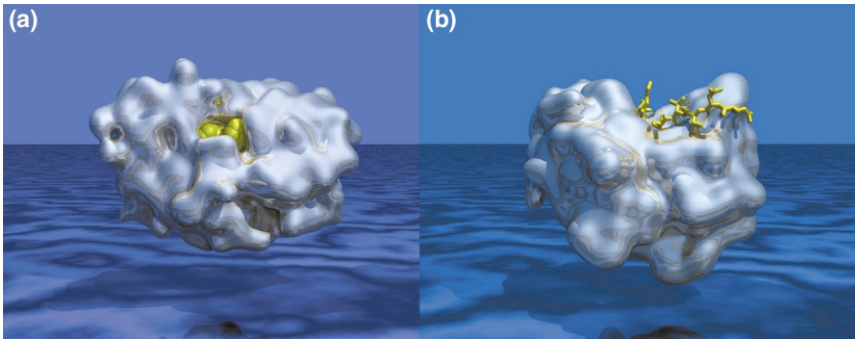

Figure 1. The crystal structures of **(a)** HIV protease and **(b)** hepatitis C virus (HCV) protease. The key differences between the surface of the active sites of the two proteases are clear. HIV protease has a deep binding-pocket, in which the ligand has a tight interaction; however, the binding site of HCV is shallow, making it a difficult enzyme target challenge. Figure kindly supplied by Vertex Pharmaceuticals (Cambridge, MA, USA).

Other research

Recently, data from a clinical trial that is thought to be the first to use a monoclonal antibody in the treatment of hepatitis C has been announced by XTL Biopharmaceuticals (Rehovot, Israel). The data was from Phase Ia trials of XTL002, an anti-hepatitis C drug, which are scheduled to move into Phase Ib later this year. The study consisted of 15 patients, who were given a single dose of the compound. Following treatment, the viral loads were reduced by 2–100-fold in half of the patients and XTL002 was well tolerated. Results from these trials indicate that LY570310 show good potency, bioavailabilty and pKa values. These results are consistent with those seen by XTL001, which is currently in Phase II studies for hepatitis B. Clinical trials of XTL002 for the prevention of re-infection in liver transplant patients are expected to be initiated shortly.

Implications of protease inhibition

David Barrett, Senior Research Manager at Fujisawa Pharmaceutical Company (Osaka, Japan) commented: 'If the promising early data for this novel compound translates into improved clinical efficacy and compliance in patients infected with HCV, LY570310 has the potential to contribute greatly to improved management of this disease and its associated complications. The rational design process employed by the Vertex and Lilly researchers, targeting a key protease involved in viral assembly, represents an important advance and validates further the rational approach to new drug discovery.'

Bill Atwell, Founder and Administrator of the Hepatitis C Aware Organization (Atlanta, GA, USA), commented: 'As a non-responder after 52 weeks of Rebetron™ treatment I welcome the possibility of patient trials starting by 2003. This news will generate excitement in the Hepatitis C community that has been waiting patiently for alternatives to interferon.'

Reference

1 Trepo, C. *et al.* (1996) Treatment of chronic hepatitis C: another therapeutic option. *Nephrol. Dial. Transplant* 11 (Suppl. 4) 62–64

2 Kim, J.L. *et al.* (1996) Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. *Cell* 87, 343–355