an improved treatment would be highly significant. Cytomegalovirus is a common cause of retinal damage and blindness in patients with AIDS, and COX-2 inhibitors have been shown to slow its replication *in vitro*[7].

Any NSAID-based treatment would probably be used in addition to antiviral therapy. 'The reduction in recurrences is likely to be additive to antiviral drugs', Kaufman says, 'but the work is early and much more needs to be done before we use these drugs for this purpose in humans.'

The Louisiana State University team will now focus on determining which COX-2 inhibitors are most effective against herpes reactivation, and on the efficacy of topical NSAID preparations. They will also attempt to repeat the results in other species; to date, all the work has been done in mice.

References

1 Kaufman, H.E. (2001) Can we prevent recurrence of herpes infections without antiviral drugs? Weisenfeld Award Lecture, *Association for Research in Vision and Ophthalmology Annual Meeting*, 1 May 2001, Fort Lauderdale, FL, USA

2 The Herpetic Eye Disease Study Group (1997) A controlled trial of oral acyclovir for the prevention of stromal keratitis or iritis in patients with herpes simplex viral epithelial keratitis. The epithelial keratitis trial. *Arch. Ophthalmol.* 115, 703–712

3 Wand, M. *et al.* (1999) Latanoprost and herpes simplex keratitis. *Am. J. Ophthalmol.* 127, 602–604

4 Kaufman, H.E. *et al.* (1999) Latanoprost increases the severity and recurrence of herpetic keratitis in the rabbit. *Am. J. Ophthalmol.* 127, 531–536

5 Kaufman, H.E. *et al.* (2001) Effects of topical unoprostone and latanoprost on acute and recurrent herpetic keratitis in the rabbit. *Am. J. Ophthalmol.* 131, 643–646

6 Chen, N. *et al.* (2000) NSAID treatment suppresses VSV propagation in mouse CNS. *Virology* 276, 44–51

7 Shenk, T. (2000) Use of microarrays to probe the replication and pathogenesis of human cytomegalovirus. *25th International Herpesvirus Workshop*, 3 August 2000, Portland, OR, USA

The rise and fall of Viagra

Janet Fricker, Freelance writer

New drugs for male erectile dysfunction (ED) are set to challenge the market dominance currently enjoyed by sildenafil citrate (Viagra™, Pfizer, Sandwich, UK). The new drugs, vardenafil (Bayer, Leverkusen, Germany) and Cialis™ (IC351; Eli Lilly, Indianapolis, IN, USA), both of which are phosphodiesterase-5 (PDE5) inhibitors, are currently in Phase III clinical trials and could prove more potent, and produce fewer side effects, than sildenafil.

ED is defined as the consistent inability to attain and maintain a penile erection adequate for satisfactory sexual activity. Vascular disease, diabetes, prostate surgery, psychiatric disorders and concomitant drug therapy can predispose to ED, which can be exacerbated by psychological factors. ED is widespread: in a large community-based epidemiology survey (Massachusetts Male Aging Study; MMAS), one-third of 1290 men aged 40–70 years reported having moderate or complete ED[1]. Other studies have found that ~50% of men in this age group have some degree of ED, and 30% report moderate-to-severe impotence[2].

Sildenafil

Sildenafil citrate, introduced in 1998, was considered to be a major breakthrough as the first effective oral treatment for ED. Sildenafil is an active inhibitor of cGMP-specific PDE5 – the enzyme responsible for degrading cGMP in the corpus cavernosum of the penis. This inhibition, combined with nitric oxide-mediated increased formation of cGMP resulting from sexual arousal, leads to more pronounced relaxation of the corpus cavernosum smooth muscle, leading to accumulation of blood in the sinusoids and, therefore, improved penile rigidity.

Sildenafil also exerts a significant effect on PDE6, an isoform important for phototransduction in the retina. Although sildenafil is about 4,000–10,000-fold more selective for PDE5 over PDE1–4 and PDE7, it is only approximately tenfold more selective for PDE5 over PDE6. Therefore, at clinically effective doses, sildenafil is likely to inhibit PDE6, accounting for the transient colour change in vision reported by 1 in 12 patients taking sildenafil[3].

'Genetic defects in PDE6 are the cause of autosomal recessive retinitis pigmentosa and autosomal dominant night blindness,' says Patrick Vallance, Professor of Clinical Pharmacology at University College London (London, UK). In addition, experimental lesions in PDE6 cause retinal degeneration in mice[4]. 'There is no doubt that complete loss of PDE6 is bad news for the retina. The question remains whether 10% inhibition will do any longer-term damage apart from reversible changes in colour vision. Sildenafil has just not been used for long enough to tell,' adds Vallance.

The more frequent side effects of sildenafil, such as facial flushing, headache, nasal congestion and dyspepsia[3], have

1359-6446/01/$ – see front matter PII: S1359-6446(01)01888-8

been assumed to be caused by vaso dilation produced as a consequence of blocking PDE5 in other areas of the body. If the assumption is correct, these side effects are likely to persist with new drugs (Ian Eardley; consultant urologist at St James' University Hospital, Leeds, UK).

In a study of 433 men, a median daily dose of 100 mg sildenafil produced an erection suitable for intercourse in 67.6% of subjects. However, psychogenic ED proved more responsive to sildenafil than ED ascribed to organic factors[3].

'Sildenafil is not effective in ~30% of patients, particularly those who have damage to the nerves of the penis either after prostatectomy, or as a result of diabetes. The hope is that the new drugs will produce higher response rates in such groups,' says Eardley.

The challenge for pharmaceutical companies has been to design new PDE5 inhibitors with increased potency and greater selectivity for PDE5 over other PDE isoenzymes. Although vardenafil and Cialis are leading the way, it is estimated that at least five other PDE5 inhibitors are in preclinical stages of development.

Drug development

Vardenafil

In a new conscious-rabbit model of ED, candidate PDE5 inhibitors were infused in the presence of the nitric oxide donor, sodium nitroprusside, and the resulting length of the rabbit's penis was used as a measurement of drug efficacy[5]. Using these screens, vardenafil was selected for efficacy and was found to be 257- and 224-fold more selective for PDE5 over PDE1 and PDE6, respectively. In comparative experiments with sildenafil, vardenafil demonstrated a 42-fold increased selectivity over sildenafil for the PDE5 enzyme over PDE1 and PDE6. *In vitro* studies also demonstrated the superior potency of vardenafil – 1 mg of vardenafil was sufficient to cause the same level of PDE5 inhibition as 9 mg of sildenafil (Bayer press release, 9 April 2001).

Phase II clinical trials data have continued to show promise for vardenafil. The results of a 12-week Phase II study in 601 men[6] found that >80% of patients taking 20 mg vardenafil reported an improvement in the quality of erection, with 74.6% of these patients experiencing successful intercourse with ejaculation. At 10 mg and 5 mg doses, 76% and 66% of patients, respectively, had improvements in the quality of erection, compared with 30% on placebo.

'The ability to achieve complete sexual intercourse with ejaculation is much more meaningful to a man with erectile function difficulties than statistics relating to erection alone,' said Hartmut Porst, lead investigator of the study and a member of the International Vardenafil Study Group.

Results from three separate subanalyses of the data[7] showed that vardenafil improves erectile function regardless of age, disease severity or cause of the problem. Results of an additional subanalysis of the Phase II study[7] demonstrated that vardenafil not only improves erections, but also positively impacts other important measurements of sexual function, including orgasmic function and intercourse satisfaction. Furthermore, these improvements were maintained consistently over a 12-week period.

The first Phase III results for vardenafil showed significant improvement in erectile function over 12 weeks in 452 men with diabetes. Mean International Index of Erectile Function (IIEF) scores for patients in the 10 mg group increased from a baseline of 11.5 to 17.0, whereas the placebo group increased to 12.6. In the 20 mg group, 72% of participants reported a significant improvement in erections compared with 13% in the placebo group ($p < 0.0001$).

Cialis

Cialis is highly selective for PDE5, with >700-fold selectivity over PDE6 and >10,000-fold selectivity over all other PDEs tested[8]. The first data in a Phase III study of 216 diabetic men[9] show that when Cialis was taken on demand in 20 mg doses for 12 weeks, 64% reported improved erections. Cialis appears to differ from both sildenafil and vardenafil in its duration of action, with a half-life of ~17 h (Christian Steif, Hanover, Germany) compared with ~4 h for vardenafil and sildenafil.

'The idea that a drug that can be taken on Friday evening and last until Sunday is attractive to some since it overcomes issues of spontaneity. But the other side of the coin is that you will have side effects for that length of time. Although people have tolerated headaches and flushing with sildenafil, they may find them more troublesome over longer periods,' says Eardley. He is also concerned that Cialis would produce longer-lasting changes in the cGMP secondary messenger system that plays an important signalling role. 'We just don't know how safe that would be,' he adds.

Apomorphine

Apomorphine hydrochloride (TAP Holdings, Deerfield, IL, USA) is a new centrally acting oral therapy that was approved by the European Regulatory authorities in January 2001, and is due to be launched soon. This drug is a dopamine-receptor agonist that works by stimulating dopamine receptors in the paraventricular nucleus, augmenting the normal neural signals initiating erection. In a Phase III trial of 854 patients with moderate and severe grades of ED, 54.4% of men given 4 mg apomorphine produced erections that enabled intercourse, compared with 33.8% taking placebo[9].

Conclusions

'It is important to have drugs with different mechanisms of action because they could benefit different patients. Furthermore, if they can be used together they might produce a synergistic response. However, the down-side is

that there could also be more side effects,' says Eardley.

References

1 Feldman, H. *et al.* (1994) Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *J. Urol.* 151, 54–61

2 Goldstein, I. and Beavo, J.A. (1998) Oral sildenafil in the treatment of erectile dysfunction. *New Engl. J. Med.* 338, 1397–1404

3 McMahon, C.G. *et al.* (2000) Efficacy, safety and patient acceptance of sildenafil citrate as treatment for ED. *J. Urol.* 164, 1192–1196

4 Beavo, J.A. (1995) Cyclic nucleotide phosphodiesterases; functional implications of multiple isoforms. *Physiol. Rev.* 75, 725–747

5 Bischoff, E. and Schneider, K. (2000) A conscious rabbit model is able to demonstrate the efficacy of vardenafil and sildenafil on penile erection. *Int. J. Impot. Res.* 12 (Suppl. 3), S65

6 Porst, H. (2001) Evaluation of vardenafil, a new highly selective PDE5 inhibitor, in a double-blind, placebo-controlled study in 601 patients with erectile dysfunction. *Congress of the International Society of Impotence Research*, 27–29 November 2001, Perth, Australia

7 Porst, H. *et al.* (2001) Vardenafil, a new highly selective PDE5 inhibitor, improves erectile function irrespective of the baseline severity and etiology of ED or age of patient. *16th Annual Congress of the European Association of Urology*, 7–10 April 2001, Geneva, Switzerland

8 Sanez de Tejada, I. *et al.* (2001) The effect of on-demand Cialis™ (IC351) treatment of erectile dysfunction in men with diabetes. *16th Annual Congress of the European Association of Urology*, 7–10 April 2001, Geneva, Switzerland

9 Heaton, J.P. (2000) Apomorphine: an update of clinical trial results. *Int. J. Impot. Res.* 12 (Suppl. 4), S67–S73

Algal compound could reverse multidrug resistance in cancer

David Bradley, Freelance writer (http://www.sciencebase.com or http://www.acdlabs.com/webzine/)

A total synthesis of a naturally occurring compound has been devised that could lead to a way of reversing drug resistance in tumour cells[1]. The development of multidrug resistance (MDR) in tumour cells is commonly observed in cell cultures and, explains pharmacologist Charles Smith of Pennsylvania State University (Hershey, PA, USA), is likely to be a major factor in limiting the clinical success of many anticancer drugs. A major mechanism through which MDR develops is the overexpression of membrane transport proteins: this process simply removes the anticancer drug from the tumour cell, preventing it from accumulating in the cell and so ultimately rendering the therapy ineffective despite its cytotoxicity.

Transporter proteins

Perhaps the most well known transporter is the transmembrane protein P-glycoprotein (P-gp), which controls the traffic of a diverse range of compounds. It is a plasma-membrane-associated, energy-dependent efflux pump and among the compounds transported are the anthracyclines, the vinca alkaloids, paclitaxel (Taxol) and certain antibiotics.

A second transporter, MDR-related protein 1 (MRP1), is also involved in the emergence of resistance to anticancer drugs and both P-gp and MRP1 can be overexpressed in the tumour cells of chemotherapy patients. 'P-gp is actually commonly overexpressed in tumours of patients after chemotherapy,' explains Smith. 'However, the data on expression of MRP1 are much more ambiguous. Although many tumours do express MRP1, there is usually no overexpression of this transporter.' The development of adjuncts to cancer treatments that can side-step these transporter proteins and enable anticancer agents to accumulate at their target sites are being keenly sought. One such compound that could lead to a solution is the natural product dendroamide A (Fig. 1).

Dendroamide A is one of three cyclic hexapeptides discovered by Smith's team in 1996 from the terrestrial blue-green alga (cyanobacterium) *Stigonema dendroideum* Fremy. It has the ability to reverse multidrug resistance in several cancers *in vitro*, such as leukaemias and breast and kidney carcinomas, at non-cytotoxic doses. Smith, who holds a patent on the compound, and his team determined the gross structures of dendroamides A–C using NMR and MS analyses[2]. The absolute stereochemistry was determined by Marfey analysis and chiral GC–MS of the derivatives.

Totally synthesized

Smith believes that the fact that dendroamide is a modified cyclic peptide makes it an amenable starting point for the synthesis of a variety of analogues, which might be further investigated for structure–activity relationships. He and his post-doctoral research colleague, chemist Zuping Xia, have now developed a total synthesis that will facilitate this process.

The total synthesis of most natural products generally involves a reverse

1359-6446/01/$ – see front matter PII: S1359-6446(01)01886-4