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Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction

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Sildenafil (Viagra™, UK-92,480) is a novel oral agent under development for the treatment of penile erectile dysfunction. Erection is dependent on nitric oxide and its second messenger, cyclic guanosine monophosphate (cGMP). However, the relative importance of phosphodiesterase (PDE) isozymes is not clear. We have identified both cGMP- and cyclic adenosine monophosphate-specific phosphodiesterases (PDEs) in human corpora cavernosa *in vitro*. The main PDE activity in this tissue was due to PDE5, with PDE2 and 3 also identified. Sildenafil is a selective inhibitor of PDE5 with a mean IC_{50} of 0.0039 μM. In human volunteers, we have shown sildenafil to have suitable pharmacokinetic and pharmacodynamic properties (rapid absorption, relatively short half-life, no significant effect on heart rate and blood pressure) for an oral agent to be taken, as required, prior to sexual activity. Moreover, in a clinical study of 12 patients with erectile dysfunction without an established organic cause, we have shown sildenafil to enhance the erectile response (duration and rigidity of erection) to visual sexual stimulation, thus highlighting the important role of PDE5 in human penile erection. Sildenafil holds promise as a new effective oral treatment for penile erectile dysfunction.

Keywords: penile erectile dysfunction; sildenafil; oral treatment; phosphodiesterase type 5

Introduction

Penile erectile dysfunction is a common medical disorder. It has an estimated prevalence of 2% in men aged 40 years, which increases to over 50% in men over the age of 70 years.[1] Penile erectile dysfunction has been defined as 'the inability to achieve and/or sustain an erection for satisfactory sexual performance'.[2] Generally, it is accepted that this disorder adversely affects quality of life. Patients often report increasing anxiety, loss of self-esteem, lack of self-confidence, tension and difficulty in the relationship with their partner.[2]

Penile erection is a haemodynamic event which is dependent upon relaxation of the smooth muscle cells of the corpus cavernosum and of its associated arterioles, with consequential increase in arterial flow into the trabecular spaces of the corpora cavernosa.[3,4] The increased blood flow causes the lacunar spaces or sinusoids to become distended which results in compression of the small venules between the sinusoids and the tunica albuginea. The relative indistensibility of the tunica albuginea results in a veno-occlusive effect such that the penile pressure increases to approach mean arterial pressure and penile rigidity develops.

There is now ample evidence from both animal experiments and *in vitro* studies with human tissue to suggest that relaxation of the smooth muscle of the corpora cavernosa is mediated by nitric oxide via cyclic guanosine monophosphate (cGMP).[5–8] During sexual stimulation, nitric oxide is released from nerve endings and endothelial cells. Nitric oxide then stimulates the cytosolic enzyme guanylate cyclase to produce cGMP which results in a decrease in intracellular calcium and allows relaxation of smooth muscle cells. Cyclic nucleotide phosphodiesterase (PDE) isozymes, which are distributed in various tissues, specifically hydrolyse cyclic nucleotides, such as cGMP.[9] Therefore, a pharmacological agent which inhibits the cGMP-specific phosphodiesterase isozyme, should enhance the action of nitric oxide/cGMP on penile erectile activity and have the potential to enhance penile erections during sexual stimulation.

To date, pharmacological therapy for penile erectile dysfunction has been largely based on the use of intracavernosal injections of vasoactive agents. Though efficacious, this form of therapy is associated with a high dropout rate for a variety

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opment of sildenafil (Viagra™; 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo [4,3-d] pyrimidin-5-yl) phenylsulphonyl]-4-methyl-piperazine, Pfizer Central Research) a novel, orally-efficacious drug for the treatment of penile erectile dysfunction.

This review focuses on *in vitro* studies relating to the mode of action of sildenafil, pharmacokinetic studies in human volunteers, and an early clinical study in patients with erectile dysfunction.

Methods

Isolation of soluble phosphodiesterase activities from human tissues

Frozen human corpus cavernosal tissue (obtained from IIAM, Exton, Pennsylvania; donor age range 48–64 years) was thawed on ice, coarsely chopped, and then homogenised in approximately 4 volumes of ice cold HEPES buffer (20 mM, containing 0.25 M sucrose, 1 mM EDTA, 1 mM phenylmethyl sulphonylfluoride [PMSF], pH 7.2) using an Ultra Turrax homogeniser at high setting. The homogenate was filtered through two layers of surgical gauze to remove any undispersed tissue and fibrous material. The filtrate was centrifuged at 100 000 g for 60 min at 4°C. The supernatant was filtered through a 0.2 μM filter and either used directly (see below) or stored in liquid nitrogen prior to analysis. A similar procedure was used for preparation of soluble fractions from samples of human cardiac ventricle and human skeletal muscle (also obtained from IIAM).

Phosphodiesterase activities in the soluble fractions prepared from human tissues were separated using a Pharmacia FPLC system (Pharmacia Ltd, Milton Keynes, UK) with a Mono Q anion exchange column (1 mL bed volume, Pharmacia Ltd). The Mono Q column was pre-equilibrated with HEPES buffer (20 mM, containing 1 mM EDTA, 0.5 mM PMSF, pH 7.2), before loading tissue soluble fractions (2–5 mL). The column was then washed with 5 ml of the buffer and the phosphodiesterase isozymes were eluted using a continuous gradient of 0–500 mM NaCl in the same buffer (total volume 55 mL) at a flow rate of 1 mL/min, and 2 mL fractions were collected. Fractions comprising the main peaks of phosphodiesterase activity were pooled and stored at −80°C for use in characterisation and inhibition studies.

Determination of phosphodiesterase activity and isozyme characterisation

The cyclic nucleotide phosphodiesterase activity in FPLC fractions was determined using a modification of the two step radioisotopic procedure of Thompson and Appleman.[11] The reaction mixture (total volume 100 μL) contained column fraction (10 to 25 μL), [^{3}H]-cGMP or [^{3}H]-cAMP (500 nM, 2 μCi/mL), bovine serum albumin (0.5 mg/mL) and $MgCl_2$ (5 mM) in Tris HCl buffer (15 mM, pH 7.4). Reaction was initiated by addition of the radiolabelled substrate, and the samples incubated in a water bath at 30°C for 30 min. The reaction was stopped by immersing the sample tubes in boiling water for 2 min. The 5′-mononucletides formed by hydrolysis of cyclic nucleotides were determined after their further conversion to nucleosides using snake venom (*Ophiophagus hannah*) nucleotidase activity as described by Thompson and Appleman.[11]

For inhibitor studies, test compounds were added to incubation mixtures in dimethyl sulphoxide (DMSO, final concentration 2% v/v). The hydrolysis of cyclic nucleotides did not exceed 15% and under these conditions, product formation increased linearly with time and amount of enzyme. IC_{50} values were determined from sigmoidal curves, fitted to plots of enzyme activity vs log compound concentration using a curve fitting programme.[12]

Phosphodiesterase activities in FPLC fractions were characterised based on their substrate specificities, effects of calcium calmodulin, the effect of cGMP on cAMP hydrolytic activity and the inhibitory potency of known selective inhibitors of the PDE isozymes (Table 1).[13–16] Human cardiac ventricle was used as a source of soluble PDE1 and human skeletal muscle (gluteus maximus) for PDE4.

Table 1 Classification of PDE isozymes[13]

Isozyme family	*Substrate specificity*	*Effect of cGMP on cAMP hydrolysis*	*Effect of calcium calmodulin*	*Standard inhibitor*
PDE 1	cAMP/cGMP	N.A.	stimulation	vinpocetine[16]
PDE 2	cAMP/cGMP	stimulation	no effect	none
PDE 3	cAMP	inhibition	no effect	milrinone[16]
PDE 4	cAMP	no effect	no effect	rolipram[16]
PDE 5	cGMP	N.A.	no effect	zaprinast[16], E4021[15]

N.A. = not appropriate for characterisation of these isozyme families
PDE = phosphodiesterases

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Pharmacokinetic studies

The following studies have been conducted to investigate the single oral dose pharmacokinetics of sildenafil.

(i) A single-blind, escalating single oral dose study, in which solution doses ranging from 1.25 to 90 mg were administered to two groups ($n = 9$ and $n = 10$) of healthy male volunteers, with random insertion of placebo. Each subject received three active doses and placebo.

(ii) An extension of the first study in which a group of 10 healthy male volunteers received single oral solution doses of sildenafil (100, 150 and 200 mg) with random insertion of placebo.

(iii) An open, randomised, two-way cross-over study to investigate the pharmacokinetics of sildenafil after oral administration (50 mg capsule) and intravenous administration (50 mg) to a group of 12 healthy male volunteers.

In all studies blood samples were taken pre-dose and at intervals up to 72 h post-dose for determination of the plasma concentrations of sildenafil.

Plasma and urine samples were assayed using a sensitive and specific HPLC assay following solid phase extraction.

Clinical study

The efficacy of sildenafil on penile erectile activity was evaluated in 12 patients who had a history of penile erectile dysfunction of at least six months duration. On clinical evaluation there was no obvious organic cause for penile erectile dysfunction. Patients were excluded from the study if there was evidence of neurovascular disease on clinical evaluation, diabetes, drug or alcohol abuse, or other established causes for their penile erectile dysfunction.

The study was a double-blind, placebo-controlled, randomised, four-way crossover design. A period of at least three days was allowed between consecutive treatment periods to ensure that there was adequate clearance of sildenafil from the circulation. On each dosing period, patients were admitted to a hospital bed with complete privacy. They received a single dose of sildenafil (10, 25 or 50 mg) or placebo. Each dose was followed by visual sexual stimulation (VSS) starting 30 min post-dose and lasting for two hours. VSS was provided by viewing of sexually explicit material chosen from a selection of videos and magazines. Drug efficacy on penile erectile activity was evaluated by measurement of penile rigidity at the base and tip of the penis by penile plethysmography (RigiScan, Dacomed Corpora-

Mean duration of rigidity of greater than 60% at the base and tip of the penis was analysed by analysis of variance (ANOVA).

Results

Phosphodiesterase activities in human corpus cavernosum

Two major peaks demonstrating cGMP hydrolytic activity and one peak demonstrating cAMP hydrolytic activity were resolved by anion exchange chromatographic analysis of soluble fractions from human corpus cavernosum (Figure 1). Peak 1, which had the greatest PDE activity, was found in soluble fractions prepared from three separate samples of human corpus cavernosum. The PDE activity in the peak was specific for cGMP as substrate and unaffected by calcium calmodulin. In

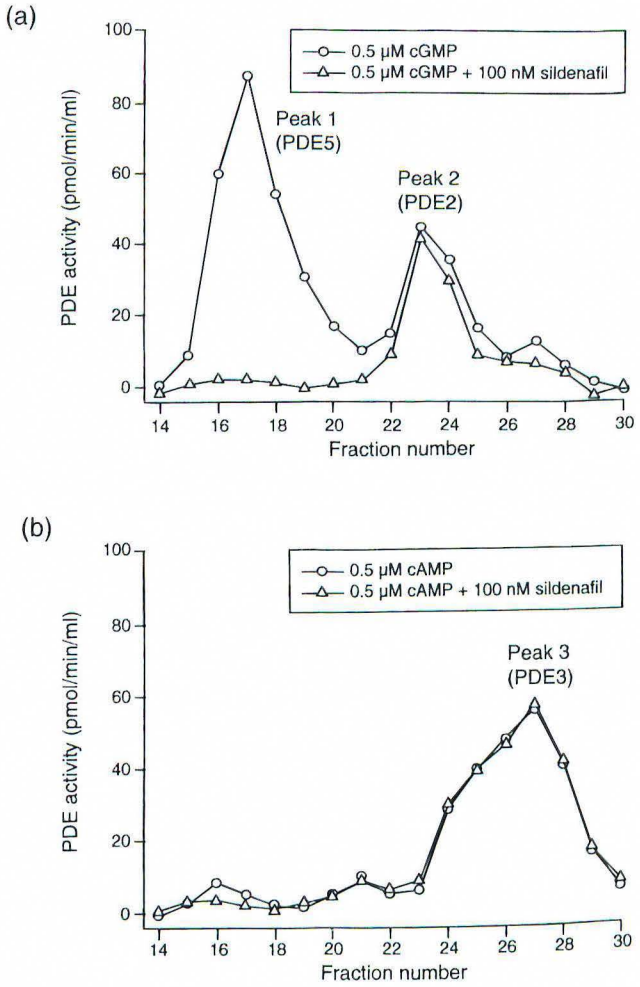

Figure 1 Separation of PDE isoenzymes from human corpus cavernosum by fast protein liquid chromatography on a Mono-Q column. Panel (a) shows hydrolysis of cGMP (0.5 μM) in the presence and absence of 100 nm sildenafil. Panel (b)

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addition it was inhibited by zaprinast, a standard PDE5 inhibitor with a mean $IC_{50} \pm SEM$ of $0.86 \pm 0.11\,\mu M$ ($n = 4$), which is similar to values previously reported for PDE5 from rat lung ($0.76\,\mu M$)[17] and porcine aorta ($0.51\,\mu M$).[15] These data indicated that peak 1 comprised predominantly PDE5. Peak 2 and peak 3 were identified as PDE2 and PDE3, respectively.

Effects of sildenafil on human phosphodiesterase activities

Sildenafil (100 nM) completely inhibited the cGMP hydrolytic activity of the PDE5 peak from human corpus cavernosum, but had no significant effect of the PDE2 or PDE3 peaks (Figure 1). Moreover, sildenafil was found to be a potent inhibitor of PDE5 activity from human corpus cavernosum with a mean IC_{50} of $0.0039\,\mu M$ (Table 2). Sildenafil had low activity against PDE2 and PDE3 from corpus cavernosum and PDE4 from skeletal muscle (IC_{50} values $>7.3\,\mu M$; Table 2). It had moderate activity against PDE1 from human cardiac ventricle (IC_{50}, $0.29\,\mu M$). These data showed that sildenafil was at lest 70-fold selective for PDE5 relative to isozymes from PDE families 1–4.

Pharmacokinetics of sildenafil in human volunteers

The results from the single-blind escalating single oral dose (studies [i] and [ii] under pharmacokinetic method) demonstrate that sildenafil is rapidly absorbed with maximum observed plasma concentrations occurring within one hour after oral dosing. Plasma concentrations decline in a bi-exponential manner with a mean terminal half life of 3 to 5 h. Pharmacokinetic simulations predict no significant accumulation of the drug after repeated once-daily dosing.

The pharmacokinetics of sildenafil were approximately dose proportional after administration of solution formulations in the range 1.25 mg to 200 mg. The maximum plasma concentration and the area under the plasma concentration–time curve increased linearly with dose. (Figures 2 and 3).

The absolute bioavailability (study[iii]above) revealed that sildenafil has a mean plasma clearance of 41 L/h and a mean steady state volume of distribution of 105 L. The mean absolute bioavailability after oral dosing of a 50 mg capsule was 41%.

There were no clinically significant effects on pulse rate, blood pressure and laboratory safety tests (haematology and biochemistry profiles) following administration of single oral doses of up to 200 mg to healthy volunteers. The main adverse events reported following doses of 90 mg and

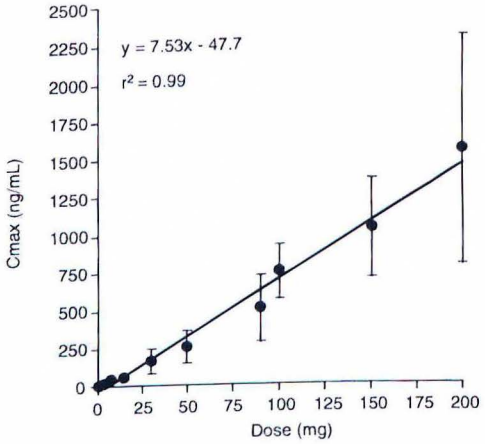

Figure 2 Mean maximum plasma concentration values (C_{max}) of sildenafil following single oral administration to healthy male volunteers. Each point represents mean $\pm$ SD from 9 or 10 subjects

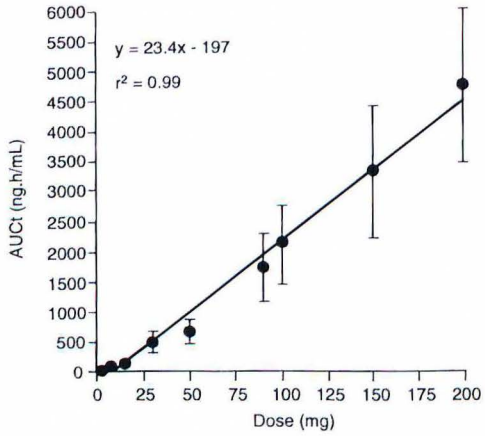

Figure 3 Mean area under the plasma concentration – time curve (AUC) values for sildenafil following single oral administration to healthy male volunteers. Each point represents mean $\pm$ SD from 9 or 10 subjects

Table 2 TC_{50} values for inhibition of human PDE isozymes by sildenafil. PDE2, 3 and 5 were isolated from corpus cavernosum, PDE1 from cardiac ventricle and PDE4 from skeletal muscle. IC_{50} values were determined using cGMP (0.5 μM) as substrate for PDE1, 2 and 5 and cAMP (0.5 μM) as substrate for PDE3 and 4. Data shown are means $\pm$ S.E.M.

Compound	IC_{50} (μM)				
	PDE1	*PDE2*	*PDE3*	*PDE4*	*PDE5*
Sildenafil	0.29 ± 0.03 ($n = 7$)	>30 ($n = 4$)	17 ± 3 ($n = 4$)	7.3 ± 0.8 ($n = 3$)	0.0039 ± 0.009 ($n = 15$)

PDE = phosphodiesterases

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above were headache and facial flushing. These adverse events were of mild to moderate severity and resolved spontaneously after a few hours. There were no treatment-related discontinuations.

Clinical efficacy

For the 12 patients entering the clinical study, the mean age was 48 years (range 36 to 63) and the mean duration of penile erectile dysfunction 3.4 years (range 1.5 to 10).

Two patients were excluded from the ANOVA analysis of mean duration of rigidity of greater than 60% at the base and tip of the penis. One patient had an erection of greater than 60% rigidity which started prior to VSS in the period when he received 25 mg of sildenafil. The second patient was excluded because penile plethysmography was not recorded due to technical difficulties.

The duration of rigidity of greater than 60% at the base and tip of the penis during VSS was significantly higher in each treatment group compared with placebo (Figure 4). The geometric mean duration (in min) of rigidity of greater than 60% at the base of the penis was 3.2 (95% confidence interval 1.1 to 7.9) on placebo, 25.9 (11.7 to 56.8, $P = 0.001$) on 10 mg, 24.1 (10.3 to 55.8, $P = 0.003$) on 25 mg and 31.8 (14.4 to 69.6, $P = 0.001$) on 50 mg of sildenafil. The corresponding values at the tip of the penis were 3.0 (95% confidence interval 1.3 to 6.4) on placebo, 19.1 (9.8 to 36.8, $P = 0.001$) on 10 mg, 26.3 (13.0 to 52.7, $P = 0.001$) on 25 mg and 26.5 (13.7 to 50.8, $P = 0.001$) on 50 mg of sildenafil. The onset of penile tumescence in all the patients was within the first few minutes of commencing VSS or approximately 30 to 40 min post-dosing with sildenafil, corresponding approximately with the peak plasma concentration of the compound.

Four patients (two at 25 mg and two at 50 mg) reported mild headache. There was no discontinuation from the study due to these adverse events, which were mild and transient. Importantly, there were no significant changes in pulse rate, blood pressure or laboratory safety data in these patients.

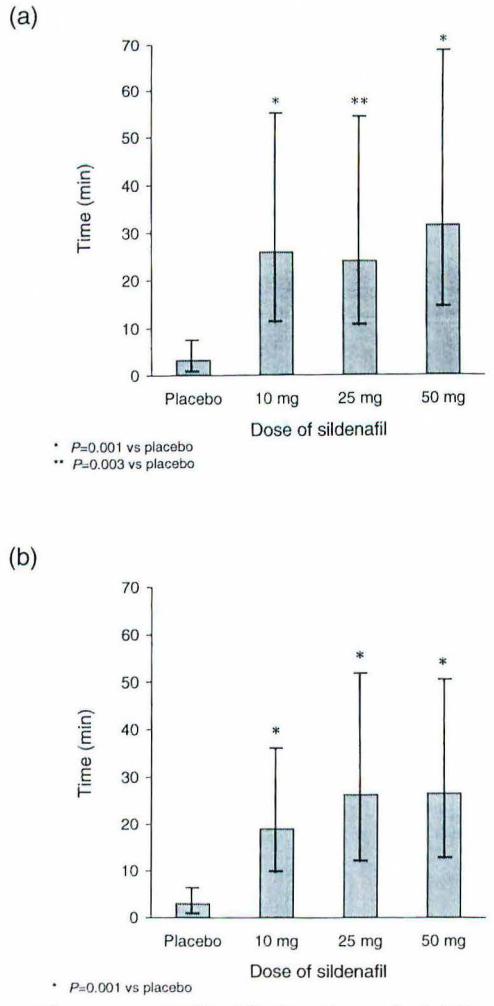

Figure 4 The mean (95% CI) duration of rigidity >60% during VSS (a) at base and (b) at tip of penis in patients who

Discussion and conclusion

During recent years it has become increasingly clear that the nitric oxide-cGMP system plays a key role in the local mechanism of penile erection.[5–8] We have demonstrated the presence of PDE2, 3 and 5 in human corpora cavernosa, and shown that sildenafil is a potent selective inhibitor of human PDE5. Furthermore, the clinical data obtained with sildenafil highlight the important role of PDE5 in human penile erection.

Our finding that cGMP-specific PDE5 was the major cGMP phosphodiesterase activity isolated from human corpus cavernosum and that cGMP-inhibited PDE3 was also present is in agreement with the report of Stieff *et al.*[18] In general, tissues such as corpus cavernosal smooth muscle, in which cGMP and cAMP appear to have similar physiological effects, express PDE3 in addition to the cGMP-specific phosophodiesterase.[19,20] This allows cGMP and cAMP to work synergistically in the tissue.[20] We have previously demonstrated that sildenafil potentiates relaxation of phenylephrine-contracted human corpus cavernosum elicited by electrical field stimulation,[21] and endothelium-dependent relaxation of rabbit cavernosal tissue induced by the muscarinic agonist, methacholine.[22] In both cases the effects of sildenafil were dependent on activation of the nitric oxide-cGMP mediated relaxation pathway. This may explain why Stieff *et al.*[18], who examined relaxation of cavernosal tissue without stimulation by nitric oxide, found that the selective PDE5 inhibitor, zaprinast, was a weaker and less effective relaxant of human cavernosal tissue than PDE3-selective agents.

By selectively inhibiting the type 5 cGMP-

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