CENTER FOR DRUG EVALUATION AND RESEARCH

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PHARMACOLOGY REVIEWS

OVERVIEW PGS 1-25

NDA #20-895

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

VIAGRA® Tablets (Sildenafil Citrate)

Pfizer Pharmaceutical Production Corporation
Ringaskiddy
County Cork, Ireland

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Introduction

SUBMISSION DATE: 9/29/97
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SPONSOR: Pfizer Pharmaceuticals Production Corporation
Ringaskiddy, County Cork, Ireland

DRUG:

Code Name: UK-92,480 (free base); UK-92,480-10 (citrate salt)

Generic Name: Sildenafil citrate

Trade Name: Viagra

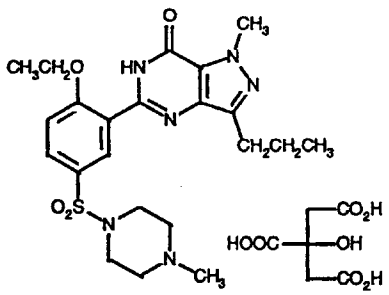
Chemical Name: 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulfonyl]-4-methylpiperazine citrate salt

CAS Registry Number: 171,599-83-0

Molecular Formula: $C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$

Molecular Weight: 666.7 (citrate salt)

Structure:



PHARMACOLOGIC CLASS: Cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) inhibitor.

PROPOSED CLINICAL INDICATION: Treatment of male erectile dysfunction

FORMULATION AND DOSE: 25 mg, 50 mg and 100 mg tablets of sildenafil are adjusted according to potency of sildenafil. The formulation per 25 mg tablet in mg/unit is as follows: 35.12 mg sildenafil citrate (based on a potency of 71.2%); microcrystalline cellulose NF; dibasic calcium phosphate; croscarmellose sodium; magnesium stearate; Blue (contains: , purified water. The proposed dose is 50 mg taken about 60 min before sexual activity. The dose may be increased to 100 mg or decreased to 25 mg. The maximum recommended human dose (MRHD) is 100 mg (= 1.4 mg/kg based on a 70 kg man).

ROUTE OF ADMINISTRATION: Oral

RATIONALE: The physiological mechanism responsible for erection of the penis involves the release of nitric oxide (NO) from nerve endings and endothelial cells in the corpus cavernosum during sexual stimulation. Nitric oxide then activates the enzyme guanylate cyclase, which results in increased levels of cyclic guanosine monophosphate (cGMP). cGMP produces vascular smooth muscle relaxation in the corpus cavernosum and causes an increase in penile blood flow and an erection. This sinusoidal engorgement works to maintain an erection by inhibiting venous return from the penis by compressing the veins responsible for draining the corpus cavernosum.

Sildenafil is a potent and selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE5). PDE5 is responsible for degradation of cGMP in the corpus cavernosum. When the NO/cGMP pathway is activated during sexual stimulation, inhibition of PDE5 by sildenafil results in increased levels of cGMP in the corpus cavernosum and increased relaxation of corpus cavernosal smooth muscle cells in response to sexual stimulation. This causes an increase in penile blood flow and an erection. Thus, sexual stimulation is required for an erection while sildenafil helps maintain one.

Sildenafil has between an 80- and nearly 20,000-fold selectivity for PDE5 found in human corpus cavernosum compared with human PDE2, PDE3, and PDE4. Sildenafil has a 10-fold selectivity for human PDE5 over human retinal PDE6.

[Note: Because sildenafil possesses antithrombotic, antivasospactic, and coronary vasodilator properties when given intravenously (i.v.), the original indication in humans was for angina pectoris. Therefore, some of the preclinical studies in animals were performed using the i.v. route of administration.]

1. PHARMACOLOGY

1.1 Overview:

Stimulation of penile autonomic innervation releases nitric oxide which activates guanylate cyclase and enhances levels of cyclic guanosine monophosphate (cGMP). The cGMP relaxes cavernosal and vascular smooth muscle and causes tumescence. Preserving cGMP, which is normally hydrolyzed by type 5 phosphodiesterase (PDE5), might be expected to facilitate initiation and/or maintenance of erection. Sponsor has documented sildenafil's potent and selective inhibition of PDE5 - the only cGMP- specific PDE in human corpora cavernosa - and propose the agent for treatment of erectile dysfunction.

Sponsor identified the dose-response for sildenafil's relatively specific cavernosal effects *in vitro* (3-300nM) and *in vivo* (10-300 µg/Kg iv). Since PDE5 also occurs elsewhere (platelets and skeletal, vascular, and visceral muscle) - and the other PDE isoenzymes are widely distributed - sponsor also examined general (safety) pharmacology at dosages 10 to approx. 30 X those affording PDE5 selectivity.

Mechanism of action. Six PDE subtypes have been characterized, and the relative potency of sildenafil for inhibiting each was identified. Sildenafil selectively and potently inhibited PDE5 which specifically degrades cyclic guanosine monophosphate (cGMP): for human enzymes, sildenafil had a >1000 fold selectivity for PDE5 over PDE2, PDE3, PDE4; an 80 fold selectivity over PDE1 (found in human cardiac ventricle); and about 10 fold selectivity over PDE6 (found in human retina). Accordingly, it is expected to inhibit the degradation of cGMP without affecting that of cyclic adenosine monophosphate (cAMP) in vivo. The selectivity (4,629-fold) of sildenafil for human PDE5 (IC50 = 3.5 nM) over human PDE3 (IC50 = 16.2 μM) is important given the known cardiovascular activity of PDE3 inhibitors, including intracellular cAMP- dependent proarrhythmogenicity.

Safety pharmacology: Sildenafil dose-relatedly changed the kinetics of the light response of the dog retina in situ, including slowing of the rate of hyperpolarization, at a threshold plasma level approx. 4x greater than that maximally effective on the corp. cavernosum. Such activity is consistent with the effect of sildenafil on PDE6, presence of PDE6 in the retina, and the role of cGMP in phototransduction.

In conscious dogs, no remarkable hemodynamic changes were seen at up to at least 10X blood levels achieving targeted cavernosal effects; at 30 X "therapeutic" dosages, modest changes within ± 20% occurred in cardiac output , total vascular resistance , and heart rate, with no cardiotonic activity. Lack of cardiovascular activity reflects relative absence of PDE3- blocking activity. Consistent with radioligand receptor binding studies *in vitro*, sildenafil had neither adrenergic, cholinergic, serotonergic or histaminergic blocking activity nor sympathomimetic or ganglion stimulating or blocking activity in cats, at up to 3 mg/Kg iv, i.e. at least 30X dosage effective on dog cavernosum. It did not facilitate induction of, or interfere with electroconversion of, PES-induced ventricular fibrillation in dogs at 30-100 X therapeutic iv dosage. It prolonged bleeding time in rats (+60%) and rabbits (+30%) at 0.3 -1.0 mg/Kg iv., i.e., 30-100X the iv doses active on the dog cavernosum.

Neither basal gastric acid secretion nor gastrointestinal motility were affected in the rat at up to 10 mg/Kg p.o.

A circulating metabolite (UK-103,320) identified in dog, rabbit, rat, mouse and man also showed PDE5 selectivity and, where tested, biological activity - including altered retinal response to light - similar to that of parent.

1.2. Activities related to mechanism of action.

Sponsor studied effects of sildenafil on corpus cavernosal smooth muscle *in vitro* and *in vivo*, and - given the tissue distribution of PDE isoenzymes - on the cardiovascular and gastro-intestinal systems, on bleeding and clotting times, and also on the retina as part of the pre-clinical evaluation of this agent.

1.2.1 Effect on PDE isoenzymes *in vitro*

Effects of sildenafil on each of the PDE isoenzymes was determined using human corpora cavernosa, cardiac ventricle and skeletal muscle as well as rabbit, dog and human platelets, rat diaphragm and kidney, and human, dog and rat retina . Potency and selectivity are shown in Table 1:

Table 1

The effects of sildenafil on the activities of phosphodiesterase (PDE) isoenzymes isolated from a variety of tissues and species

PDE isoenzyme	Source	n	Geometric mean IC_{50} value with 95% Confidence Interval.
PDE1	Human cardiac ventricle	6	280.0 nM (229 - 337)
	Rat kidney	2	430.0 nM *
	Rat diaphragm	5	218.0 nM (123 - 386)
PDE2	Human *corpora cavernosa*	5	68.0 mcM (31.6 - 146.3)
	Rat kidney	3	>100 mcM
	Rat diaphragm	3	32.8 mcM (18.8 - 57.2)
PDE3	Human *corpora cavernosa*	4	16.2 mcM (9.5 - 27.8)
	Human platelet	3	41.2 mcM (26.1 - 65.0)
	Rabbit platelet	3	48.0 mcM (24.0 - 98.0)
PDE4	Human skeletal muscle	3	7.2 mcM (4.5 - 11.5)
	Rat kidney	1	19.0 mcM *
	Rat diaphragm	3	6.3 mcM (5.3 - 7.5)
PDE5	Human *corpora cavernosa*	15	3.5 nM (2.5 - 4.8)
	Human platelet	3	6.1 nM (3.0 - 12.6)
	Rabbit platelet	4	3.9 nM (3.6 - 4.1)
	Dog platelet	1	4.8 nM *
	Rat diaphragm	5	1.8 nM (0.7 - 4.6)
PDE6	Human retina - cone	6	34.1 nM (24.5 - 47.4)
	Human retina - rod	6	37.5 nM (29.0 - 48.5)
	Dog retina - cone	11	26.9 nM (19.4 - 37.1)
	Dog retina - rod	9	58.2 nM (46.1 - 73.4)
	Rat retina - cone	4	26.9 nM (17.2 - 42.2)
	Rat retina - rod	5	67.4 nM (56.3 - 80.8)

n = Number of samples or experiments
IC_{50} = Concentration required to inhibit enzyme activity by 50%

* Results are geometric means, except for values where n=2 which are arithmetic means

Sildenafil selectively, and potently, inhibited PDE5 in human and other tissue with mean IC50 values ranging from ca. 2 to 6 nM. Retinal PDE6 is also susceptible, being blocked at approx. 10-fold higher concentrations. In contrast , sildenafil exhibited only modest activity against the cGMP-degrading calcium/calmodulin- dependent PDE1 found in human cardiac ventricles and the indicated rat tissues. That is, sildenafil exhibited ca. 80-fold greater potency for the human PDE5 compared with human and rat PDE1 isoenzymes. Consistent with demonstrating preferential activity against cGMP hydrolyzing PDE enzymes, sildenafil weakly inhibited the cGMP-stimulated PDE2 isoenzyme isolated from human corpora cavernosa (IC50 68.0 μM) and rat diaphragm and rat kidney (IC_{50}s 32.8 and >100 μM, respectively). Likewise, sildenafil is a weak inhibitor of cGMP-inhibited PDE3 found in the human corpora cavernosa (IC_{50} 16.2 μM) and human (IC_{50} 41.

μM) and rabbit (48.0 μM) platelets. Sildenafil only weakly inhibited the cAMP specific PDE4 isoenzyme found in human skeletal muscle (IC_{50} 7.2 μM) and rat kidney (IC_{50} 19.0 μM) and rat diaphragm (IC_{50} 6.3 μM). Accordingly, for human enzymes, sildenafil has a >1000 fold selectivity for PDE5 over PDE2, PDE3, PDE4; an 80-fold selectivity over cardiac ventricular PDE1; and the least selectivity (approx. 10-fold) vs. retinal PDE6.

1.2.2 Functional effects in *Corpus Cavernosum*.

Sildenafil dose-relatedly enhanced the relaxant effect of tissue NO released by electrical stimulation, methacholine, and/or sodium nitroprusside in isolated rabbit and human cavernosal strips. It had no direct relaxing activity in either rabbit or human cavernosal smooth muscle. Intravenously, it potently enhanced the tumescent
(i.e., cavernosal pressor) effect of pelvic nerve stimulation or intracavernosal sod. nitroprusside injection at dosages devoid of appreciable cardiovascular effect:

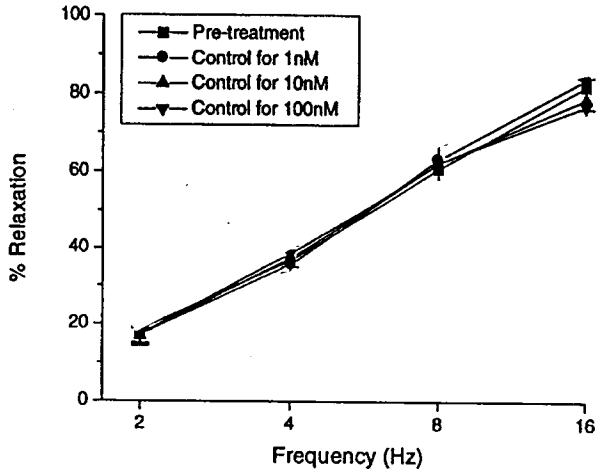
1.2.2.1 *In Vitro assays*

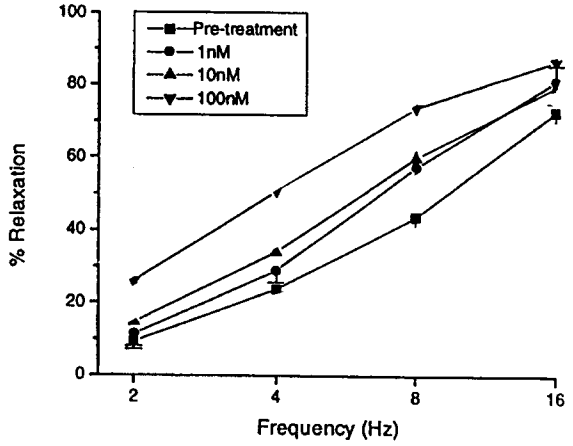
1.2.2.1.1. Rabbit:

In an assay of rabbit cavernosal strips pre-contracted with phenylephrine and made to relax with NO/cGMP-dependent stimuli , i.e. electrical field stimulation (EFS), sod. nitroprusside, or methacholine , sildenafil significantly and dose-relatedly enhanced the relaxant effect of such stimuli: threshold and maximum observed effect occurred at sildenafil levels of <10nM and 100 nM, respectively (Fig.1; Table 2).

Figure 1

The effects of sildenafil (Lower Panel) and vehicle (Upper Panel) on the relaxation of phenylephrine (10 mcM)-contracted rabbit isolated *corpus cavernosal* strips induced by electrical field stimulation (EFS: 2, 4, 8 and 16 Hz, 0.2 msec, 10 V for 10 sec).





Each point represents the mean, ± S.E.M., of the results obtained from 8 to 9 strips.

Parallel straight lines were fitted to percentage relaxation *vs* log frequency plots for each tissue and the significance of the differences in intercepts at 2Hz between pre-treatment and treatment responses were examined using Student's t-test for paired data. For the treated tissues (Lower Panel), the differences between the EFS responses for 1, 10 and 100 nM sildenafil treatments and pre-treatment were significant; $P<0.05$, $P<0.01$, $P<0.001$, respectively. For the vehicle-treated (time-matched control) tissues there were no significant differences between the lines.

Vehicle	=	0.6% v/v lactic acid, 50 mcl
Pre-treatment	=	Responses prior to addition of sildenafil or vehicle
%		Percentage

Table 2

The effects of sildenafil on the relaxation of phenylephrine (10 mcM)-contracted rabbit isolated *corpus cavernosal* strips induced by sodium nitroprusside (SNP).

Treatment	n	Mean, (95% Confidence Interval) IC_{50}s for SNP (mcM)	Treatment	n	Mean, (95% Confidence Interval) IC_{50}s for SNP (mcM)
Pre-treatment	10	6.35 (3.41 - 11.83)	Pre-treatment	10	6.15 (3.04 - 12.46)
Time matched control	8	12.36 (7.46 - 20.47)	Sildenafil 10 nM	10	4.94 (3.24 - 7.53)**
Time matched control	10	10.39 (6.34 - 17.04)	Sildenafil 100 nM	10	1.07 (0.74 - 1.56)***
Time matched control	8	9.77 (6.16 - 15.50)	Sildenafil 1 mcM	7	0.77 (0.44 - 1.36)***

n = Number of tissues
Vehicle = 0.6 % v/v lactic acid, 50 mcl
Pre-treatment = Responses prior to addition of sildenafil or vehicle
IC_{50} = Concentration required to produce a 50% relaxation of the phenylephrine-induced contraction

Significance of difference from time-matched control ** P <0.01, ***P<0.001
(Analysis of Covariance adjusted for pre-treatment values)

The effects of sildenafil on the relaxation of phenylephrine (10 mcM)-contracted rabbit isolated *corpus cavernosal* strips induced by methacholine.

Treatment	n	Mean, (95% Confidence Intervals) IC_{50}s for methacholine (nM)	Treatment	n	Mean, (95% Confidence Intervals) IC_{50}s for methacholine (nM)
Time matched control	6	38.0 (21.4 - 67.6)	Sildenafil 1 nM	6	23.4 (10.5 - 53.7)
Time matched control	7	49.0 (30.2 - 77.6)	Sildenafil 10 nM	7	20.0 (9.3 -41.7)*
Time matched control	7	40.7 (27.5 - 60.3)	Sildenafil 100 nM	7	11.7 (6.3 - 21.9)**
Time matched control	7	43.7 (29.5 - 64.6)	Sildenafil 1 mcM	7	9.8 (5.6 - 16.2)***

n = Number of tissues
Vehicle = 0.6 % v/v lactic acid, 50 mcl
IC_{50} = Concentration required to produce a 50% relaxation of the phenylephrine-induced contraction

Significance of difference from time-matched control *P<0.05, ** P <0.01, ***P<0.001
(Student's Independent t-test)

At higher concentrations (IC50 = ca. 500 nM) sildenafil *per se* also relaxed such tissue - an effect completely prevented by the NO synthase inhibitor L-n- nitroarginine. Again, endogenous NO/cGMP is implicated as a sildenafil mechanism. The non-selective PDE inhibitor papaverine relaxed this tissue even in the presence of L-n- nitroarginine.
Accordingly, sildenafil relaxes corpus cavernosal smooth muscle *in vitro* by enhancing the effect of dilators mobilizing the endogenous NO/cGMP cascade. Sildenafil 100 nM caused a near maximal effect since 1 μM did not cause a marked further potentiation of EFS-, SNP- or methacholine-induced relaxation.

1.2.2.1.2. Human:

Sildenafil also potentiated relaxation of pre-contracted human isolated corpora cavernosa induced by EFS in a dose-related manner and over a similar concentration range active on rabbit isolated corpora cavernosa (ca. 1-100 nM).As in the rabbit, the electrical stimulus relaxes via endogenous NO release. Again, sildenafil had no direct muscle relaxant activity.

1.2.2.2. *In situ assays* : intracavernosal pressure of anesthetized dogs.

1.2.2.2.1. Pelvic nerve stimulation:

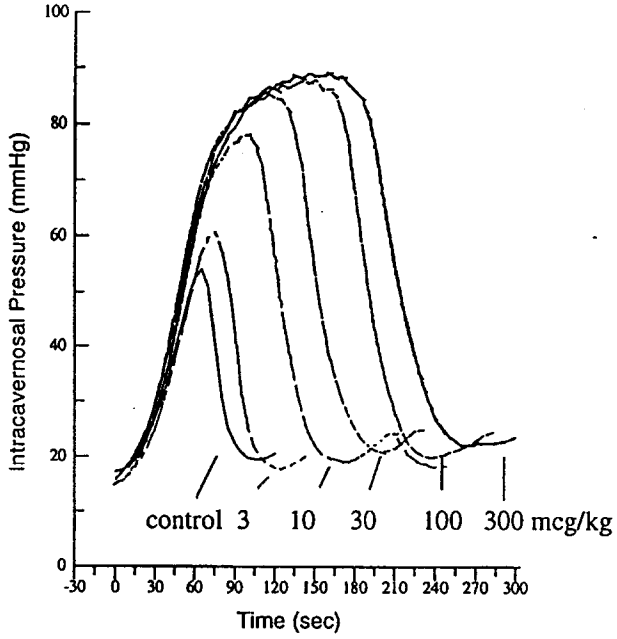
Sildenafil enhanced the increase in intracavernosal pressure elicited by stimulation of the pelvic nerve. A cavernosal pressure of ca. 30% of mean blood pressure achieved by electrical stimulation *per se* (which releases NO) was promoted, dose-relatedly, to up to 70% of systemic blood pressure by pre-treatment with 10 to 100 µg/Kg iv. of sildenafil. Blood sildenafil levels averaged 14 and 49 ng/ml after 30 and 100 µg/Kg dosages, respectively. Over this dose range, mean arterial BP, HR, and LV pressure were not appreciably affected. LV dP/dT index of contractility, elevated approx. 10% at all dosages, was, accordingly, not dose-relatedly or appreciably affected.

1.2.2.2.2. Intracavernosal sod. nitroprusside (SNP):

Injection of SNP (1 to 5 µg) into the corpora cavernosa produced an initial transient fall in pressure (<10 mm Hg) followed by a slowly developing rise which returned to baseline within 5 min. or less. Sildenafil (3 to 300 µg/kg iv.) increased the magnitude and duration of the tumescent response to SNP in a dose-related manner and the mean dose (95% CI) required to produce a response 50% of the maximum was 16.2 (11.1 - 23.6) µg/kg iv.(figure 2.).

Figure 2

A representative figure showing the effects on intracavernosal pressure of sodium nitroprusside (SNP) injected into the *corpus cavernosum* either alone (control) or following intravenous doses of sildenafil in the anaesthetised dog.



Neither blood pressure nor heart rate of these anesthetized dogs was appreciably affected over this dose range. Thus 10-300 μ g/Kg of sildenafil selectively enhances cavernosal sensitivity to purinergic (i.e., cGMp-mediated) stimuli.

In vitro and *in vivo* studies *in toto* indicate that sildenafil enhances NO→cGMP stimulation of the corpus cavernosum rather than acting as a direct smooth muscle relaxant.

1.2.3 Functional effects on other tissues expressing PDE5 enzyme.

In addition to being located in human corpora cavernosa, PDE5 occurs in platelets and muscle (vascular; skeletal; gastrointestinal). Functional consequences of blocking the enzyme in such tissues were investigated *in vitro* and *in vivo*::

1.2.3.1 Platelet aggregation/disaggregation

Sponsor assayed effect of sildenafil alone and combined with the NO donor SNP on platelet disaggregation *in vitro* and on agonist-induced platelet aggregation *in vitro* and *ex vivo* . Platelet rich plasma (PRP) prepared from rabbit and human blood was used. Sildenafil *per se* had no effect on agonist-induced platelet aggregation or on platelet disaggregation, but potentiated the platelet anti-aggregatory and disaggregatory effects of SNP *in vitro* and *ex vivo*. The *ex vivo* effect was maintained on sub-acute (5 days) dosing and there was no evidence of tachyphylaxis or the development of tolerance.

1.2.3.1.1. *In vitro* studies on rabbit and human platelet aggregation :

1.2.3.1.1.1. Rabbit:

Sildenafil alone, at a concentration of 1 μM, had no effect on agonist-induced (e.g. PAF) platelet aggregation but did significantly potentiate the platelet anti-aggregatory activity of 3 μM SNP (a NO donor) against 5 different thrombotic stimuli (Table 3).

Table 3

Effects of sildenafil alone and in combination with sodium nitroprusside (SNP) on platelet aggregation produced by a variety of agonists in rabbit platelet rich plasma (PRP).

Agonist and concentration	Mean, ± S.E.M., percentage platelet aggregation		Mean, ± S.E.M., percentage inhibition of platelet aggregation by 3 mcM SNP	
	Vehicle	Sildenafil 1 mcM	Vehicle	Sildenafil 1 mcM
PAF 10 nM	59.8 ± 4.4 (n=4)	60.8 ± 2.0 (n=3)	47.7 ± 1.6 (n=4)	75.7 ± 1.5** (n=3)
Collagen 6 mcg/ml	75.3 ± 1.8 (n=3)	71.8 ± 2.4 (n=3)	41.8 ± 1.5 (n=3)	98.5 ± 1.5** (n=3)
ADP 3 mcM	47.5 ± 2.5 (n=3)	46.0 ± 2.2 (n=3)	48.0 ± 0.6 (n=3)	89.0 ± 0.6** (n=3)
U46619 3 mcM	70.3 ± 0.5 (n=3)	68.3 ± 2.3 (n=3)	56.5 ± 3.3 (n=3)	94.2 ± 0.4** (n=3)
A23187 3 mcM	68.0 ± 6.6 (n=4)	71.5 ± 3.9 (n=3)	31.7 ± 3.4 (n=4)	55.7 ± 1.9** (n=3)

n = Number of animals
Vehicle = 10 mM HCl for aggregation studies
Vehicle = Distilled water for inhibition of aggregation studies
PAF = Platelet activating factor
ADP = Adenosine diphosphate
U46619 = Thromboxane A_2 receptor agonist
A23187 = Calcium ionophore

Significance of difference from vehicle group ** $P<0.01$
(Student's independent t-test)

The potentiating activity of sildenafil to enhance both anti-aggregatory and disaggregatory of SNP on ADP- aggregated platelet -rich plasma was concentration- related over the 10 nM to 1μM range. In further studies, 1 μM sildenafil alone had no platelet anti-aggregatory or disaggregatory activity but significantly reduced mean IC50 and EC50 for SNP-induced platelet anti-aggregation and disaggregation, respectively, from 4.61 to 0.59 μM and from 23.23 to 1.59 μM, respectively., i.e., by about an order of magnitude.

1.2.3.1.1.2. Human:

Sponsor studied platelet- rich human blood aggregated with ADP. Over the concentration range 0.3 to 30 μM, ADP induced a concentration-related aggregation of human platelets, the EC50 being 1.6 μM. This action of ADP was not affected by sildenafil 1 μM. However, the

compound significantly reduced the mean IC50 for SNP-induced platelet anti-aggregation from 2.4 to 0.8 μM.
The dose-response for ability of sildenafil to enhance SNP was investigated in human PRP aggregated with 20 μM ADP. In human PRP, 20 and 40 ng/ml sildenafil (equivalent to ca 40 and 80 nM, respectively) potentiated the platelet anti-aggregatory effects of SNP compared with the vehicle (distilled water). The mean (95% CI) shifts in the SNP IC50 were 1.7 (1.4 - 2.1)-fold and 3.0 (2.7 - 3.3)-fold for 20 and 40 ng/ml, respectively. Functional activity in human platelets was comparable with that observed in rabbit platelets. Accordingly, sildenafil *per se* had no effect on aggregation of either human or rabbit platelets provoked by ADP, or provoked by any of 4 other thrombotic stimuli - including PAF or collagen - in rabbits. However, the compound consistently and significantly potentiated the platelet anti-aggregatory effects of the NO donor SNP *in vitro* (rabbit and man) and ex vivo (rabbit: see below).

1.2.3.1.2. *Ex vivo* studies on rabbit platelet aggregation

1.2.3.1.2.1. Acute intravenous administration:

Effects on the platelet anti-aggregatory activity of SNP were studied using platelet rich plasma (PRP) prepared from blood of anesthetized rabbits which had been treated with the compound. In these experiments, PRP was aggregated with 4 μM ADP in the presence and absence of SNP. Sildenafil potentiated the anti-aggregatory effects of SNP measured ex vivo: In PRP obtained from anaesthetized rabbits treated with 0.1 mg/kg sildenafil iv., the mean (±S.E.M) IC_{50} for the anti-aggregatory effects of SNP was significantly reduced from the control value of 9.98 ± 1.49 to 2.80 ±0.39 μM in samples taken 30 min. after sildenafil administration . In samples taken 120 min. after dosing, the IC_{50} (5.64 ± 0.56 μM) was still significantly reduced . These results indicate that a single iv. dose of sildenafil (0.1 mg/Kg) can appreciably potentiate the anti-aggregatory effect of SNP in the rabbit, and with a duration of action consistent with its plasma half-life (approximately 1h) in this species.

1.2.3.1.2.2. Sub-acute(6 days) oral dosing: rabbit

Study protocol was similar to that of the acute iv. study except rabbits were dosed bid for 6 days at 1 mg/Kg , blood was drawn 2 hr. after the first dose on day 1 and 6, and the SNP-potentiating activity of sildenafil compared for any evidence of tolerance . In the day 1 and day 6 vehicle-treated groups, the geometric mean (95% CI) IC_{50}s for the anti-aggregatory effect of SNP were almost the same - 7.2 (4.0 - 13.0) and 7.0 (3.7 - 13.3) μM, respectively. In the sildenafil-treated groups, the IC_{50}s for SNP for days 1 and 6 were 1.7 (0.9 - 3.3) and 1.2 (0.7 - 1.8) μM, respectively. The IC_{50}s in the drug-treated groups were significantly different from their respective vehicle controls.

Thus, the ability of sildenafil to potentiate the platelet anti-aggregatory activity of SNP was maintained following 5 days b.i.d. dosing, with no evidence of tachyphylaxis or tolerance. - at least of a high 1 mg/Kg dose.

1.2.3.2 Antithrombotic activity:

Because sildenafil enhanced platelet anti- aggregatory effect of sod. nitroprusside *in vitro* and *ex vivo* , effects on thrombus formation were assessed in a small *in vivo* study of four anesthetized rabbits with stenosed damaged l. carotid arteries. Blood flow through such arteries is subject to cyclic flow reductions; such blood flow variations are said to reflect platelet thrombus formation. Results: Doses of 0.01 and 0.03 mg/Kg iv., and 1, 3, and 10 mg/Kg i.d. were said to afford, at the higher doses, complete reversal of the cyclical flow variations in 1 to 3 of the four rabbits tested, accompanied, however, by 5 to 20 mm Hg reductions in mean blood pressure. Since this

was a pilot study, full dose response and comprehensive hemodynamic data are lacking and the results are only suggestive.

1.2.3.3. Bleeding and clotting time:

1.2.3.3.1. Rat:

Effect on bleeding time was assessed 15 min. post-dose using the rat tail bleeding method. Clotting times were measured 20 to 25 min. post-dose using blood collected from a cannulated jugular vein. At 0.1 mg/kg iv., sildenafil did not affect either bleeding or clotting time. Increasing the dose to 0.3 mg/kg iv. increased the bleeding time by approximately 60%, although the change was not statistically significant, without appreciably affecting the clotting time. The positive control (aspirin :10 mg/kg iv.), as expected from its known pharmacological properties, markedly prolonged the bleeding time, and produced a modest (approximately 30%) increase in the clotting time. Thus, sildenafil did not affect blood clotting time at 0.1 and 0.3 mg/kg iv. but did increase bleeding time at the higher dose although less than that observed after aspirin.

1.2.3.3.2. Rabbit:

The effects of sildenafil, heparin and aspirin on bleeding time were assessed 20, 40 and 60 min. post-dose using the ear of anesthetized rabbits and a Simplate bleeding time device. Sildenafil (1 mg/kg iv.) and heparin (150 IU/kg iv.), significantly prolonged the bleeding time with mean increases of 51 ± 8 and 102 ± 17 sec, respectively (control ≡ 120 sec.) The combination of sildenafil and heparin had a greater effect than either agent alone but the increase (145 ± 37 sec) was additive rather than synergistic and there was no evidence of an interaction between the two agents. Aspirin alone, at 1 and 10 mg/kg iv. (equivalent to clinically-used low and high dose aspirin, respectively), tended to slightly prolong bleeding time, although the effect was not significant. The combination of sildenafil with each of the 2 doses of aspirin also did not significantly affect bleeding time when compared with sildenafil alone. Consequently, there was no evidence of an interaction between the 2 agents.

Accordingly, a high intravenous dose of sildenafil increased bleeding time in the rabbit ear preparation. This effect was additive with that of heparin, and there was no evidence of an interaction between sildenafil and heparin - or aspirin. The dose which prolonged bleeding time (1 mg/Kg) was 33 to 100 -fold greater than an anti-thrombotic dose (0.01-0.03 mg/Kg iv.) in that species , and 10 to 100 X greater than the doses (10-100 µg/Kg iv.) producing a targeted cavernosal effect in the dog. A similar trend was observed in the rat at a 3-fold lower dose (0.3 mg/Kg), but the compound had no effect on blood clotting times in this species. Effects are consistent with the identified actions of sildenafil on platelets in the absence of an effect on the clotting cascade.

1.2.3.4 Cardiovascular effects:

Effects of sildenafil on vascular smooth muscle, which contains PDE5, and on the intact cardiovascular system were investigated using both *in vitro* and *in vivo* models. These include isolated vascular preparations (e.g. canine coronary artery, rabbit aorta), dog cardiac trabeculae, conscious Okamoto spontaneously hypertensive rats (SHR) and hemodynamic studies in anesthetized and intact conscious dogs.

1.2.3.4.1. Isolated artery (dog; rabbit)

cGMP content: In canine isolated coronary artery sections, incubation for 5 min. with sildenafil (10 and 100 nM) increased the tissue levels of cGMP, but not cAMP, in a concentration-dependent manner up to approx. 4x basal levels.

Contractility of rabbit aorta: Aorta rings were contracted with phenylephrine (PE); EC50 value was 0.33 μM (95% CI: 0.20 - 0.52). Sildenafil (1 μM) *per se* had no effect on the PE concentration-contraction curve , but the PE curve was shifted to the right by 1 μM glyceryl trinitrate (GTN) to a PE EC50 value of 1.65 (1.05 - 2.59). A combination of GTN (1 μM) and sildenafil (1 μM) markedly increased the EC50 for phenylephrine to 6.85 (4.33 - 10.84). Comparison of the ratio of EC50 values for vehicle vs. sildenafil (1.36) and vehicle vs. GTN (5.0) alone, and vehicle vs. sildenafil + GTN (20.8) suggests that the actions of sildenafil and GTN are synergistic rather than additive. Accordingly, sildenafil enhances the relaxation of vascular smooth muscle *in vitro* provoked by guanylate cyclase/cGMP (recruited in these studies by GTN), but shows no direct activity.

Contractility of rabbit coronary artery:
Sponsor measured the perfusion pressure in rabbit Langendorff hearts with air embolus-damaged coronary arteries to determine whether sildenafil could antagonize endothelin (ET-1)- induced vasoconstriction. Sildenafil produced significant concentration-related inhibitions of the response to ET-1 ranging from 14 ± 4% at 100 nM to 64 ± 5% at 30 μM. Accordingly, sildenafil does not appreciably block contractions of isolated vascular smooth muscle produced by ET-1, or PE.

1.2.3.4.2. Dog cardiac trabeculae:

Sponsor compared effects of isoprenaline (β -agonist), sildenafil (up to 10 μM), and the selective PDE3 inhibitor milrinone on tension generated by field-stimulated isolated cardiac (presumably l. ventricular) trabeculae carnae. Milrinone (0.1 to 60 μM)increased the developed tension up to a level equivalent to 61% of the isoprenaline maximum response. In contrast, sildenafil had no effect on the contractility of the trabeculae up to a concentration (10 μM) which is 100-300 fold higher than that which inhibits PDE5 in the isolated human and rabbit corpus cavernosum (section 1.1.2.1 above). Absence of positive inotropic activity is consistent with it's weak PDE3- inhibiting activity (i.e., IC50 of ca. 30 μM in human cavernosal and platelet tissue).

1.2.3.4.3. Effect on blood pressure of spontaneously hypertensive (SH) rat:

At doses (0.03 and 0.1 mg/Kg iv.) which are active on the corpus cavernosum in the anaesthetized dog i.e., which enhance the cavernosal pressor response to pelvic nerve stimulation, sildenafil had no effect on blood pressure and heart rate in SHR. However, higher iv. doses (0.3 and 1.0 mg/Kg) as well as a 10 mg / Kg oral dose did decrease blood pressure by approx. 25 mm Hg without any consistent dose-related tachycardia. Antihypertensive effect of the oral dose persisted for at least 3 hours. Such vasodepressor activity is consistent with vasodilatation resulting from a cGMP enhancement in vascular smooth muscle and, therefore, from the known actions of sildenafil.

1.2.3.4.4. Hemodynamic effects in conscious normotensive dogs.

In a study of 5-6 conscious instrumented dogs, a variety of hemodynamic variables were monitored along with plasma drug levels following oral administration. A dose of 0.1 mg/Kg, which afforded a mean sildenafil plasma level of 13.8 ng/ml, did not provoke any remarkable hemodynamic effect in the conscious dog. Higher doses (0.3; 1.0 mg/Kg) achieving plasma levels of 34 to 115 ng/ml produced only modest effects (e.g., slight changes in heart rate, LVEDP, and LV dP/dT) consistent with vasodilation and reductions in cardiac preload and/or afterload resulting from cGMP enhancement in vascular smooth muscle. Another study at up to 3 mg/Kg (plasma drug levels not monitored, but max. of 350 ng/ml expected) revealed very modest changes of up to only 10-20% in a variety of parameters including total systemic vasc. resistance(- 15%), cardiac output (+ 16%), LV dP/dT (+ 5%), and heart rate(+ 25%); QT interval decreased by only 16 msec.

As noted above in dog pelvic nerve stimulation studies, the threshold plasma sildenafil level for concentration-related cavernosal effects was approx. 5-15 ng/ml with near maximal effect at ca. 50 ng/ml. Accordingly, there are no outstanding or unexpected hemodynamic changes in dogs at up to at least 10 X the blood levels achieving desired cavernosal effects in that species.

1.2.3.4.5. Hemodynamic effects in atropinized and ganglion/ adrenoceptor-blocked dogs.

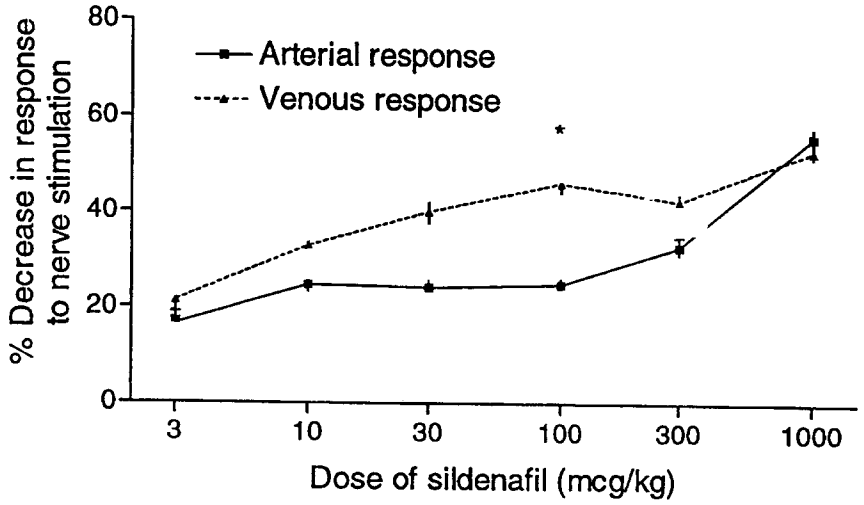
In 4 dogs treated with sildenafil and compared with time-related control animals (n=3), a statistically significant reduction in a standard noradrenaline pressor response (ca. 100 mm Hg) of 19.2 ± 0.5 mm Hg was noted 5 min. after administration of 3 mg/kg sildenafil iv. The pressor response to noradrenaline returned with time but was still significantly reduced up to 2.5 h post-dose (7.5 ± 1.7 mm Hg). This duration of action is consistent with the plasma half-life in the dog of 5.2 h (see Metabolism and Pharmacokinetics Section). In the ganglion- and α/β -adrenoceptor-blocked dog, there was no evidence of a direct effect of sildenafil on heart rate. In the single dog dosed with 10 mg/kg iv., MABP fell acutely by 33 mm Hg, but heart rate increased by only 2 bpm. Likewise, in the 4 dogs given 3 mg/kg, MABP fell by a mean of 36 ± 3.9 mm Hg, while the heart rate increased by only 2 to 3 bpm. Accordingly, high sildenafil doses produced marked falls in blood pressure in pharmacologically sympathectomized animals with no tachycardic effect. The PDE-3 blocker milrinone was not tested in this model for corresponding activities.

1.2.3.4.6. Arterio-venous dilator activity in the anesthetized dog.

Sponsor determined the arterio-venous balance of sildenafil 's vasodilator activity vis a vis the mixed nitrate (isosorbide dinitrate, ISDN) and arterial calcium antagonist (diltiazem) classes of vasodilator. The arterial and venous responses to electrical stimulation of the lumbar sympathetic chains in perfused arterial and venous segments in the hind limbs of the anaesthetized, β-adrenoceptor-blocked, atropinised dog were used in this assessment: Sildenafil displayed dose-related arterio-venous dilator properties (Fig.3) at intravenous doses (3-300 μg/Kg) similar to those active on the corpus cavernosum in the anaesthetized dog (10-100 μg/Kg). Its arterio-venous profile resembled that of ISDN - i.e., approx. equipotent on both arterial and venous sides .

Figure 3

The effects of intravenously-administered sildenafil on the hind limb arterial and venous vasoconstrictor responses to electrical stimulation of the lumbar sympathetic chains of β-adrenoceptor-blocked and atropinised anaesthetised dogs (n=4)



n = Number of animals
% = Percentage

Results are expressed as mean, ± S.E.M., values

Dogs were β-adrenoceptor blocked and atropinised by the intravenous administration of propranolol (2 mg/kg) and atropine (1 mg/kg)

Electrical stimulation parameters for the lumbar sympathetic chains were 3 Hz (left chain), 8 Hz (right chain), 0.5 msec, supramaximal voltage (30 V)

Significance of difference between responses * $P < 0.05$
(Student's independent t-test)

However, over the dose ranges studied, sildenafil inhibited neural vasoconstriction up to approx. 50% Vs ≥ 80% for ISDN. In this regard, both agents differed from that of the arterio-selective diltiazem which selectively blocked neural arterial vasoconstriction a max. of 100%.
g. Hemodynamic effects in anesthetized cats:
At all doses tested (0.3,1, and 3 mg/Kg iv.), sildenafil provoked marked but short-lasting (ca. 5 min.) drops in mean BP (ca. 40 %), LV systolic pressure (ca. 25%), and LV dP/dT (20%) which were stat. signif. but not dose-related, and an 18% increase in heart rate at the highest dose.

1.2.3.5 Gastro-intestinal effects:

1.2.3.5.1. *In vitro:*

Sponsor studied effects of sildenafil on GI tract since PDE5 and PDE1 are expressed in such smooth muscle:

Concentrations of sildenafil which were maximally effective on the corpus cavernosum in vitro (100 nM) had no effect on electrically stimulated rat esophagus, histamine-stimulated guinea

pig ileum, or carbachol-stimulated mouse ileum. However, higher concentrations (1 μM) relaxed pre-contracted mouse and rat ileum with equivalent potency. This concentration had no effect on the dog isolated lower oesophageal sphincter, although at 10 μM, sildenafil caused a significant relaxation of this preparation. The latter effect was blocked by the NO synthase inhibitor, L-nitroarginine, suggesting sildenafil acts to potentiate endogenous NO.

1.2.3.5.2. *In vivo:*

At oral doses up to and including 10 mg/kg, sildenafil did not affect basal gastric acid secretion or gastrointestinal propulsive activity in the rat. Histamine , cimetidine, and morphine behaved as expected of their known pharmacology. Consequently, the compound did not affect gastrointestinal function in controlled studies in this species.

1.2.3.6. Retinal effects:

It is reported that illuminated rhodopsin, the visual pigment of the rod, stimulates retinal PDE6 via the G-protein, transducin. As levels of cGMP decrease, cGMP-gated ion channels close and hyperpolarize photoreceptors. Hyperpolarisation induced by blue light was recorded in vitro in dark-adapted dog retina by measuring the transretinal potential via bilateral silver/silver chloride electrodes. In addition, in situ electrical activity of the dog dark-adapted retina in response to a light stimulus was detected using a corneal electrode, and the resultant electroretinogram analyzed.

Results:

Dose-related actions of sildenafil on phototransduction were detected *in vitro* (fig 4) as well as *in vivo* (fig. 5; Table 4). Such effects were observed *in vitro* at concentrations of 0.3-100 μM (IC50 = 3μM). The slowing of the rate of hyperpolarisation is consistent with the compound opposing the normal physiological response to light. This change , the slowing of the rate of repolarisation, and the change in the duration of response have all been observed with other non-selective PDE inhibitors, and is expected of sildenafil. according to sponsor.

Figure 4

A representative figure to show the effects of sildenafil on the response of the dog dark-adapted isolated retina to a 50 msec blue light challenge. Each curve represents the average response to 7 consecutive light challenges.

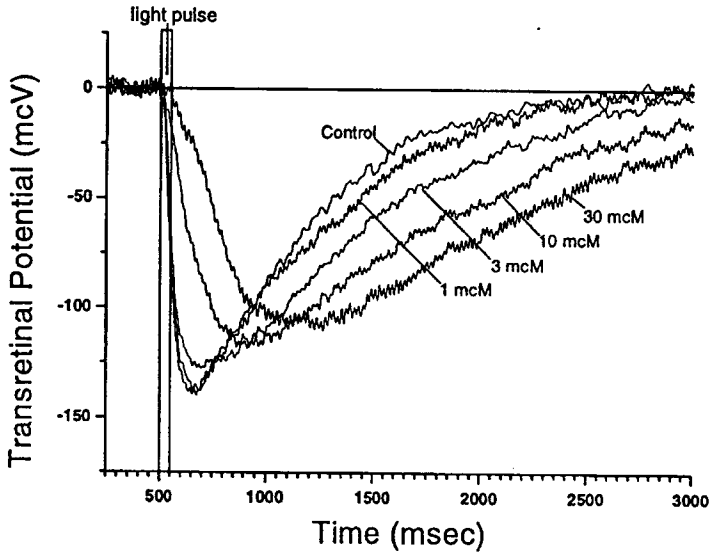
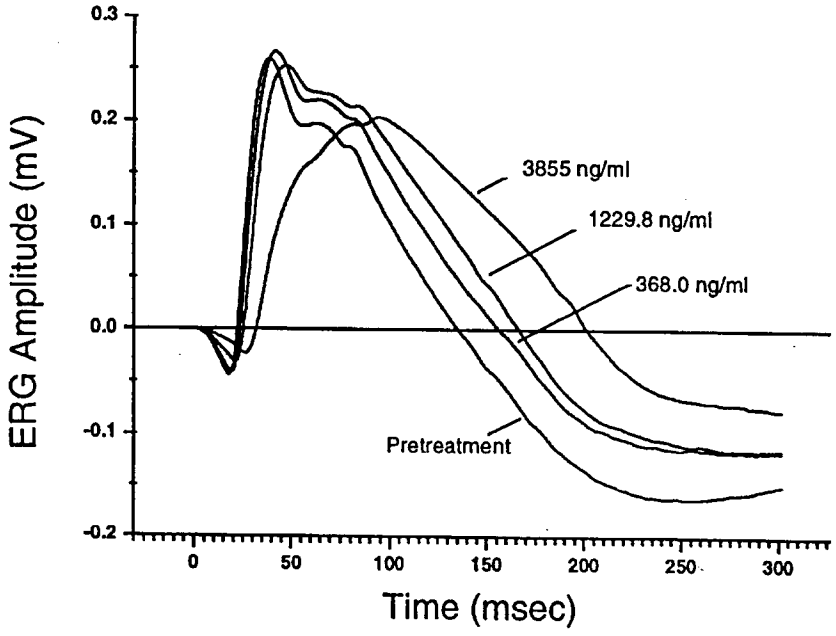


Figure 5

A representative figure to show the effects of intravenously-administered sildenafil on the electroretinogram (ERG) of the dark-adapted anaesthetised dog evoked by a flash (50 mcsec) of blue light. Each curve represents the average response to 5 consecutive light challenges.



In vivo , electroretinogram changes occurred at serum sildenafil concentrations of 368-3855 ng/ml with a suggestion that the dose-response may begin at lower threshold level of 100-200 ng/ml. Implicit times of the a- and b-waves (the most sensitive marker of effects according to sponsor) were increased by approximately 10% at a mean total plasma concentration of 368.0 ± 24.0 ng/ml. As noted above, cavernosally -active plasma levels achieved following bolus doses of 30 µg/kg iv. of sildenafil were 13.7 ± 1.2 ng/ml.
Thus, plasma concentrations which are active *in vivo* on the retina are nearly 27-fold higher than those active on the corpus cavernosum in the anaesthetized dog. However, a more conservative safety ratio (e.g., min. retinally-active conc./ max. cavernosally - active conc.) would be approx. 4 (i.e., 200 ng per ml/50 ng per ml).

Table 4

The effects of intravenously-administered sildenafil and vehicle on the electroretinogram (ERG) of the dark-adapted anaesthetised dog (n=5). The ERG was evoked using a flash (50 mcsec) of blue light.

Mean, ± S.E.M. plasma concentration (ng/ml)	a-wave amplitude (mV)		a-wave implicit time (msec)		b-wave amplitude (mV)		b-wave implicit time (msec)		Slope of the b-wave mcV/msec)	
	Sildenafil	Vehicle	Sildenafil	Vehicle	Sildenafil	Vehicle	Sildenafil	Vehicle	Sildenafil	Vehicle
Pre-dose values	-0.045 ± 0.006	-0.042 ± 0.004	+19.36 ± 0.53	+19.75 ± 0.91	+0.30 ± 0.03	+0.30 ± 0.03	+41.51 ± 1.47	+42.92 ± 1.25	+13.87 ± 1.43	+12.82 ± 1.21
Control	-2.0 ± 2.6	+12.8 ± 5.0	+1.3 ± 0.6	+0.3 ± 1.4	+3.5 ± 2.0	+3.0 ± 3.0	+0.2 ± 1.0	+1.5 ± 0.5	+4.4 ± 2.2	+1.5 ± 0.6
113.2 ± 7.6	-7.1 ± 1.6	-0.2 ± 7.7	+3.5 ± 0.6	+3.3 ± 1.2	+5.5 ± 1.5	+2.1 ± 1.5	+3.2 ± 0.3	+3.0 ± 0.7	+2.5 ± 1.1	+1.5 ± 2.3
368.0 ± 24.0	-16.2 ± 4.1	-0.7 ± 6.2	+8.9 ± 1.2	+4.7 ± 1.7	+5.8 ± 2.1	+1.5 ± 1.9	+9.5 ± 1.1	+4.8 ± 1.1	-3.0 ± 0.9	-1.0 ± 2.6
1229.8 ± 62.7	-50.5 ± 2.4	-3.9 ± 7.9	+14.1 ± 3.3	+5.4 ± 1.7	+1.2 ± 4.6	+0.9 ± 2.6	+23.4 ± 2.9	+5.8 ± 1.1	-21.9 ± 3.2	-3.4 ± 3.0
3855.0 ± 7.6	-53.5 ± 2.7	-9.2 ± 11.0	+45.3 ± 4.6	+6.7 ± 2.3	-21.0 ± 4.3	+0.1 ± 2.9	+125.6 ± 10.6	+7.5 ± 1.3	-72.9 ± 8.7	-6.2 ± 3.3

n = Number of animals
Vehicle = Acidified saline
Control = No treatment

\- = Decrease
\+ = Increase
Pre-dose values are the absolute values for each parameter expressed in the units indicated
Sildenafil was infused intravenously to achieve steady state plasma concentrations. The compound was infused at increasing concentrations (3.3 mcg/kg/min for 30 min plus 50 mcg/kg/min for the first 2 min) from 30 to 60, (10 mcg/kg/min for 30 min plus 100 mcg/kg/min for the first 2 min) from 60 to 90, (33 mcg/kg/min for 30 min plus 350 mcg/kg/min for the first 2 min) from 90 to 120 and (100 mcg/kg/min for 30 min plus 800 mcg/kg/min for the first 2 min) from 120 to 150 min.
Results are mean, ± S.E.M., percentage changes from pre-dose values based on the average of 5 consecutive ERG readings taken pre-dose and at steady state plasma levels

1.3 General / Safety Pharmacology

1.3.1 *In vitro* receptor binding profile:

Displacement of the binding of appropriate radioligands to brain tissue membranes in vitro was used as an index of affinity for adrenoceptors, adenosine (A_1 and A_2), dopamine (D_1 and D_2), histamine (H_1),5-HT_1, 5-HT_2, muscarinic and opioid receptors, dihydropyridine, verapamil and diltiazem calcium channel binding sites and benzodiazepine binding sites: At concentrations up to 10 µM, sildenafil displayed little affinity for α and β adrenoceptors, dopamine (D_1 and D_2), histamine (H_1), 5-HT_1, 5-HT_2, muscarinic and opioid receptors and verapamil and benzodiazepine binding sites (Table 5).

Table 5

Summary of radioligand binding affinity of sildenafil for receptors and binding sites *in vitro.*

Receptor type/ binding site	Standard agent	IC_{50} (nM) of:	
		Standard	Sildenafil
α_1-adrenoceptor	Prazosin	0.51 ± 0.04	>10,000
α_2-adrenoceptor	UK-14,304	1.68 ± 0.10	>10,000
β-adrenoceptor	Propranolol	4.6*	>10,000
Porcine Adenosine A_1	Cyclohexyladenosine	4.0 ± 0.3	5300 ± 1400
Rat Adenosine A_1	Cyclohexyladenosine	0.88	51% inhibition at 100 nM, 100% at 10000 nM
Porcine Adenosine A_2	Cyclopentyladenosine	1082 ± 56	300 ± 71
Human Adenosine A_{2a}	NECA	24.5	48% inhibition at 100 nM, 87% at 10000 nM
Human Adenosine A_{2b}	NECA	36.1	86% inhibition at 10000 nM
Human Adenosine A_3	NECA	16.3	32% inhibition at 10000 nM
Dopamine D_1	SCH-23,390	1.5 ± 0.2	>10,000
Dopamine D_2	Butaclamol	14.8 ± 3.0	>10,000
Histamine H_1	Pyrilamine	3.7*	>10,000
5-HT_1	5-HT	1.71 ± 0.40	>10,000
5-HT_2	Ketanserin	4.6*	>10,000
Muscarinic	Atropine	3.7*	>10,000
Opioid	Naloxone	2.7 ± 0.3	>10,000
Dihydropyridine	Nitrendipine	0.44 ± 0.11	51% inhibition at 10,000nM
Verapamil	Verapamil	405 ± 140	>10,000
Diltiazem	Diltiazem	84 ± 18	3400 ± 340
Benzodiazepine	Flunitrazepam	4.26*	>10,000

All values represent the mean, ± S.E.M., of at least 3 experiments or the average* of 2 experiments

IC_{50} = Concentration required to reduce the degree of specific binding by 50%

Weak affinity was seen at porcine adenosine A_1 receptors (IC_{50} 5.3 μM), human adenosine A_{2b} (86% inhibition at 10 μM) and A_3 (32% inhibition at 10 μM) receptors and dihydropyridine (51% inhibition at 10 μM) and diltiazem (IC_{50} 3.4 μM) binding sites. In comparison with the mean IC_{50} of 3.5 nM for inhibition of PDE5 in human corpus cavernosum, it is unlikely that these weak binding affinities have biological relevance at efficacious doses. However, sildenafil had moderate affinity for the rat adenosine A_1 (IC_{50} approximately 100 nM), porcine adenosine A_2 (IC_{50} 300 nM) and human adenosine A_{2a} (48% inhibition at 100 nM and 87% inhibition at 10 μM) receptors, although the concentrations are still 28 to 85 times higher than that required to inhibit the human PDE5 enzyme found in the corpus cavernosum .

Receptor affinity profile and selectivity is consistent with a threshold for noteworthy cardiovascular , gastrointestinal, or central effects occurring at least an order of magnitude higher dosages and/or blood levels than those achieving targeted cavernosal effects in the dog - the species in which the broadest spectrum of bioassays were performed.

1.3.2 Effects on Electroconversion in the Anaesthetized Dog.

This was one of the more important safety assays performed by the sponsor. Since enzyme and receptor selectivity is never absolute, sildenafil can block PDE3 as well as the targeted PDE5, and the PDE3 inhibitor milrinone is pro-arrhythmogenic in humans and dog models:
Effect of sildenafil on the electroconversion of ventricular fibrillation to sinus rhythm was investigated in pentobarbitone-anaesthetised dogs. Ventricular fibrillation was induced by high frequency electrical stimulation(PES) of an electrode positioned in the right ventricle, and the fibrillating hearts were cardioverted using DC shock applied through direct contact electrodes positioned on either side of the chest wall. A large bolus intravenous injection of 3 mg/kg sildenafil (N= 4 dogs) - vs. an equivalent volume of vehicle (acidified saline, pH 2.66; N= 4 dogs) - neither reduced the duration (train) of high frequency electrical stimulation required to induce ventricular fibrillation (Table 6), nor augment the energy required to defibrillate the hearts (Table 7) during the 3 hour experimental protocol.

Table 6

The effects of intravenously-administered vehicle (Panel A) and sildenafil (Panel B) on the duration (train) of high frequency electrical stimulation (40 Hz, 1 msec, 4 V) required to induce ventricular fibrillation in the hearts of pentobarbitone-anaesthetised dogs (n=4).

Panel A Vehicle (Acidified saline (mean, ± S.E.M., pH 2.66 ± 0.15), 10ml plus 2ml wash-in i.v.)

Dog number	Meaned duration (sec) control 0 to 60 min	Meaned duration (sec) 60 to 120 min	Percentage change from control	Meaned duration (sec) 120 to 180 min	Percentage change from control	Meaned duration (sec) 60 to 180 min	Percentage change from control
OFE2	0.525	0.550	+4.8	0.600	+14.3	0.575	+9.5
OFF2	0.600	0.575	-4.2	0.600	0.0	0.588	-2.0
OGC3	0.525	0.500	-4.8	0.450	-14.3	0.475	-9.5
OFC2	0.700	0.500	-28.6	0.675	-3.6	0.588	-16.0
Mean percentage change ± S.E.M.			-8.2 ± 7.1		-0.9 ± 5.9		-4.5 ± 5.5

Panel B Sildenafil (3 mg/kg i.v.)

Dog number	Meaned duration (sec) control 0 to 60 min	Meaned duration (sec) 60 to 120 min	Percentage change from control	Meaned duration (sec) 120 to 180 min	Percentage change from control	Meaned duration (sec) 60 to 180 min	Percentage change from control
OEP3	0.575	0.575	0.0	0.575	0.0	0.575	0.0
OFC4	0.600	0.475	-20.8	0.525	-12.5	0.500	-1.7
OFE1	0.575	0.525	-8.7	0.575	0.0	0.550	-4.4
OGD1	0.550	0.500	-9.1	0.425	-22.7	0.462	-16.0
Mean percentage change ± S.E.M.			-9.7 ± 4.3		-8.8 ± 5.5		-5.5 ± 3.6

\- = Decrease
\+ = Increase

Table 7

The effects of intravenously-administered vehicle (Panel A) and sildenafil (Panel B) on the energy (Joules) required to defibrillate the hearts of pentobarbitone-anaesthetised dogs (n=4).

Panel A Vehicle (Acidified saline (mean, ± S.E.M., pH 2.66 ± 0.15), 10 ml plus 2 ml wash-in i.v.)

Dog number	Meaned energy (Joules) control 0 to 60 min	Meaned energy (Joules) 60 to 120 min	Percentage change from control	Meaned energy (Joules) 120 to 180 min	Percentage change from control	Meaned energy (Joules) 60 to 180 min	Percentage change from control
OFE2	25.0	25.0	0.0	22.5	-10.0	23.8	-4.8
OFF2	25.0	22.5	-10.0	25.0	0.0	23.8	-4.8
OGC3	20.0	22.5	+12.5	22.5	+12.5	22.5	+12.5
OFC2	22.5	17.5	-22.2	22.5	0.0	20.0	-11.1
Mean percentage change ± S.E.M.			-4.9 ± 7.4		+0.6 ± 4.6		-2.0 ± 5.1

Panel B Sildenafil (3 mg/kg i.v.)

Dog number	Meaned energy (Joules) control 0 to 60 min	Meaned energy (Joules) 60 to 120 min	Percentage change from control	Meaned energy (Joules) 120 to 180 min	Percentage change from control	Meaned energy (Joules) 60 to 180 min	Percentage change from control
OEP3	20.0	25.0	+25.0	20.0	0.0	22.5	+12.5
OFC4	22.5	20.0	-11.1	22.5	0.0	21.2	-5.6
OFE1	27.5	22.5	-18.2	28.8	+4.5	25.6	-6.9
OGD1	17.5	20.0	+14.3	17.5	0.0	18.8	+7.1
Mean percentage change ± S.E.M.			+2.5 ± 10.2		+1.1 ± 1.1		+1.8 ± 4.8

\- = Decrease
\+ = Increase

Accordingly, sildenafil did not facilitate induction of fibrillation or impede cardioversion at a dosage (3 mg/Kg iv.,) approx. 30 to 100 times cavernosally active dosages (30-100 µg/Kg iv. in the same species. Unfortunately , a positive control such as the PDE3 blocker milrinone was not included in this bioassay [Milrinone was proarrhythmogenic in a dog model of PES-provoked V fib; such data has been published by it's author, Dr. Lucchesi].

1.3.3 Effects on nervous system

1.3.3.1 Autonomic nervous system.

In the standard anesthetized cat nictitating membrane preparation (electrical stimulation of the pre-ganglionic superior cervical sympathetic nerve), sildenafil (0.3, 1 and 3 mg/kg iv.) did not reveal any sympathomimetic or ganglion stimulating or blocking activity as evidenced by no change in muscle tension (basal , or neuronally stimulated).

1.3.3.2 Central nervous system

The effects of sildenafil on the central and peripheral nervous systems were assessed from acute studies of effects on the appearance and behaviour of rats and mice, the performance of mice on a rotating rod, alcohol- and pentobarbitone-induced sleeping times in mice, and analgesia assays (tail flick and acetic acid-induced abdominal constriction in mice).

Results: Behaviorally, sildenafil was tolerated by rats (up to 300 mg/Kg) and mice (up to 10 mg/Kg) following single oral and intravenous administration, respectively, and following multiple dose oral administration (30 mg/kg t.i.d./5 days) - despite peripheral erythema indicative of vasodilation. In chlorpromazine-controlled mouse studies, the compound did not a) display sedative activity; b). interact meaningfully with alcohol or pentobarbitone; or c) impair motor co-ordination (rotarod test) in the mouse at 10 mg/Kg p.o. Somatic function in the cat. Sildenafil was not analgesic in mice following multiple dosing over 5 days at 30 mg/ Kg t.i.d.

1.3.4. Hemodynamic effects:

1.3.4.1 Rats and dogs

These are described above under: Effects related to inhibition of PDE (1.2.3.4 : Cardiovascular effects)

1.3.4.2 Effects on Histaminergic, Cholinergic, and Adrenergic agonists in cat.

At up to 3 mg/Kg iv., sildenafil revealed no evidence of any consistent compound-related antagonistic effects on the cardiovascular responses to histamine, acetylcholine, phenylephrine or isoprenaline in the anesthetized cat. This is consistent with absence of any affinity of this compound - at 10 μM - for these receptor types in *in vitro* radioligand binding studies (see above 1.3.1), and with the minimal systemic hemodynamic effects at cavernosa - specific blood sildenafil levels.

1.3.5 Effects on the Gastrointestinal tract *in vivo.*

As indicated above (Effects related to inhibition of PDE : section **1.2.3.5**) sponsor reported no remarkable functional changes either *in vitro* or *in vivo* in bioassays involving rat, mouse, guinea pig, or dog .

1.3.6 Respiratory system

Activity of sildenafil, vehicle (acidified saline) and morphine on blood gas tensions and blood pH were assessed in rats implanted with arterial and venous cannulae. As expected of it's central depressant activity, morphine (4 mg/kg iv.) decreased arterial blood pH and pO2 and increased pCO2. In contrast, sildenafil (3 mg/kg iv.) did not significantly affect any marker of respiratory function in the conscious rat.

1.3.7 Renal function

The effects of sildenafil on renal function were investigated. Sildenafil, by its cGMP PDE inhibitory mechanism, increases tissue cGMP levels, and consequently may enhance effects of atrial natriuretic factor. Effects on urinary pH, excretion of fluid and electrolytes, and concentrations of electrolytes, over a 5 h period after dosing, were monitored in conscious, normotensive female rats given an oral saline load. Furosemide significantly increased the mean total urinary excretion of fluid, Na+,K+,Cl-, and significantly reduced urinary pH and concentrations of electrolytes. In contrast, sildenafil (1, 3 and 10 mg/kg p.o.) reduced urinary pH and the mean total excretions of fluid, Na+, K+ and Cl- to essentially the same extent at all dose levels, although the changes did not always reach statistical significance. Accordingly, at doses of 1 mg/kg p.o. and above, sildenafil has antidiuretic activity in the rat. In my experience, this would be expected in view of the compound's vaso-depressor activity at the dosages tested (at 10 mg/Kg,blood pressure of the SH rat was reduced by 25 mmHg for at least 3 hours: **1.2.3.4** c., above).