

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-845

PHARMACOLOGY REVIEW(S)

NDA #21-845

**REVIEW AND EVALUATION OF
PHARMACOLOGY AND TOXICOLOGY DATA**

**RevatioTM
(Sildenafil Citrate)**

**Pfizer, Inc.
New York, NY**

***Reviewer:*
Thomas Papoian, Ph.D.**

**Division of Cardio-Renal Drug Products (HFD-110)
Center for Drug Evaluation and Research
Food and Drug Administration**

May 12, 2005

PHARMACOLOGY AND TOXICOLOGY REVIEW

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1. EXECUTIVE SUMMARY

1.1. SUBMISSION BACKGROUND

This NDA proposes to use an already marketed drug (i.e., Viagra; sildenafil citrate; treatment of erectile dysfunction) for a new indication (i.e., pulmonary arterial hypertension; PAH). Several primary and secondary pharmacodynamic studies, pharmacokinetic studies, and safety pharmacology studies were submitted to support the new indication. No toxicology studies were submitted, since these have already been submitted for NDA #20-895 (Viagra), and have been cross-referenced in the current NDA.

1.2. RECOMMENDATIONS

1.2.1. *Recommendation on Approvability*

Approvable

1.2.2. *Recommendations for Additional Nonclinical Studies*

None

1.2.3. *Recommendations on Labeling*

Note: Due to the differences between the dose used for erectile dysfunction (100 mg Viagra) versus the 20 mg three times a day (t.i.d.) dosing recommended for pulmonary hypertension (60 mg/day Revatio), the Revatio draft labeling sections for (1) "Carcinogenesis, Mutagenesis, Impairment of Fertility" and (2) "Pregnancy" differ from the labeling for Viagra, specifically the dose-exposure multiples for animals relative to humans.

Recommendations on labeling:

1. Under the section "Carcinogenesis, Mutagenesis, Impairment of Fertility", the applicant uses

Levels of parent drug plus major metabolite should be used in estimating drug exposure for both male and female rats.

2. Under the sections: (a) "Carcinogenesis, Mutagenesis, Impairment of Fertility" and (b) "Pregnancy", the applicant estimates human exposure levels from a 20 mg t.i.d. (3X/day) dosing regimen by . The expected human exposure should be based on AUCs determined in studies in which subjects received 20 mg t.i.d.

3. Under the section "Pregnancy", doses in rats and rabbits are expressed as surface area-based multiples of the human dose. It is preferable to compare animal to human exposures on the basis of AUCs when that data is available.

4. Although comparisons of animal and human exposures should be made on the basis of AUCs or body surface area, animal doses expressed in mg/kg should be included whenever animal studies are described. This information was not provided for the rat carcinogenicity study.

1.3. BRIEF SUMMARY OF NONCLINICAL FINDINGS

1.3.1. *Pharmacological Activity*

Sildenafil's mechanism of action is through selective inhibition of phosphodiesterase 5 (PDE5), an enzyme responsible for the breakdown of cyclic guanosine monophosphate (cGMP). Increased cGMP levels result in vascular smooth muscle relaxation and vasodilatation. Given that nitric oxide (NO) and cGMP are involved in modulating pulmonary vascular tone, and that relatively high levels of PDE5 are found in pulmonary epithelium, the use of sildenafil to increase NO/cGMP-dependent pulmonary vasodilatation as a treatment for pulmonary arterial hypertension (PAH) is proposed.

1.3.2. *Toxicological Findings*

No toxicology studies were submitted. *In vitro* and *in vivo* toxicity studies supporting the chronic administration of sildenafil were previously submitted and reviewed under NDA #20-895 (Viagra; sildenafil citrate; Pfizer, Inc.). In those studies conducted over relatively large dose ranges, no adverse cardiovascular (including proarrhythmogenic and hemorrhagic), autonomic, cytotoxic, tumorigenic/genotoxic, or reproductive toxicities were found.

1.3.3. *Nonclinical Safety Issues Relevant for Clinical Use*

No safety issues have been identified in the current submission that were not previously addressed in NDA #20-895 (Viagra).

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2. PHARMACOLOGY AND TOXICOLOGY REVIEW

2.1. BASIC INFORMATION

NDA Number: #21-845

Date of Submission: Dec. 2, 2004

Applicant: Pfizer Inc, New York, NY

Reviewer Name: Thomas Papoian, Ph.D.

Division Name: Cardio-Renal Drug Products

HFD #: 110

Review Completion Date: May 12, 2005

Drug Product: Revatio™ Tablets

Drug Substance:

Generic Name: Sildenafil citrate

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

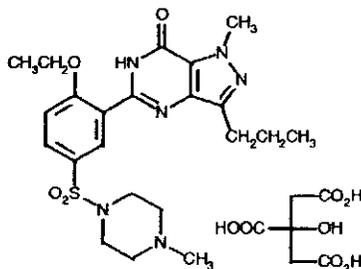
Chemical Name: 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-pyrimidin-5-yl)phenylsulfonyl]-4-methylpiperazine citrate salt

CAS Registry Number: 171,599-83-0

Molecular Formula: C₂₂H₃₀N₆O₄S · C₆H₈O₇

Molecular Weight: 666.7 (citrate salt)

Structure:



Related INDs/NDAs: IND #46,863 (Viagra; sildenafil citrate; Pfizer, Inc.); NDA #20-895 (Viagra; sildenafil citrate; Pfizer, Inc.)

Pharmacological Class: Cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) inhibitor

Clinical Indication: Treatment of pulmonary arterial hypertension (PAH)

Clinical Rationale: Sildenafil's mechanism of action is through selective inhibition of phosphodiesterase 5 (PDE5), an enzyme responsible for the breakdown of cyclic guanosine monophosphate (cGMP). Increased cGMP levels result in vascular smooth muscle relaxation and vasodilatation. Given that nitric oxide (NO) and cGMP are involved in modulating pulmonary vascular tone, and that relatively high levels of PDE5 are found in pulmonary epithelium, the use of sildenafil to increase NO/cGMP-dependent pulmonary vasodilatation as a treatment for pulmonary arterial hypertension (PAH) is proposed.

Clinical Formulation: The relative composition of Revatio™ (sildenafil citrate) 20 mg tablets is identical to that of Viagra™ (sildenafil citrate) 25, 50, and 100 mg tablets, except that the blue dye has been removed (Table P.1.1; Applicant's table):

Table P.1-1. Composition of the Medicinal Product

Component	Grade	Function	20 mg Tablet
			(mg/Unit)
Sildenafil citrate	Pfizer	Active	
Microcrystalline cellulose	NF.		
Dibasic calcium phosphate	USP.		
Croscarmellose sodium	NF.		
Magnesium stearate	NF.		
	Pfizer		
	USP.		
	Pfizer		
	USP.		
Theoretical Total Weight (mg)			

(a) Based on a theoretical potency factor of 100%

(b) The microcrystalline cellulose

Route of Administration: Oral. The recommended human dose is 20 mg three times a day (t.i.d.).

Relevant NDA Background: This NDA proposes to use an already marketed drug (sildenafil citrate) for a new indication (PAH). Several primary and secondary pharmacodynamic studies, pharmacokinetic studies, and safety pharmacology studies were submitted to support the new indication. No toxicology studies were submitted, since these were submitted for the approved

NDA #20-895 (Viagra; treatment of erectile dysfunction), and have been cross-referenced in the current NDA.

2.2. PHARMACOLOGY

2.2.1. Primary Pharmacodynamics

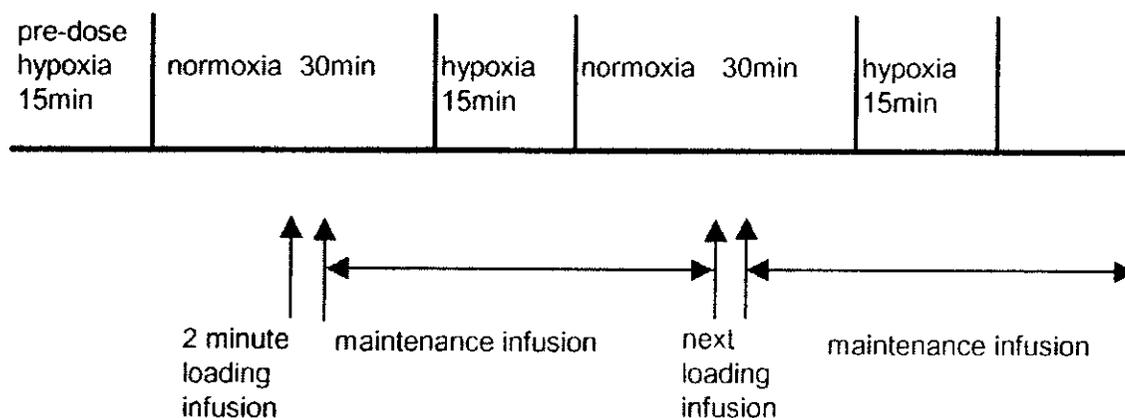
2.2.1.1. The Effect of Intravenously-Administered UK-92,480,27 on Hypoxic Pulmonary Vasoconstriction in the Anesthetized Dog

Purpose: To determine the effect of intravenously-administered sildenafil on reducing the increase in pulmonary vascular tone produced by hypoxic pulmonary vasoconstriction in anesthetized dogs.

Methods: Male and female beagle dogs (11-14 kg) were anesthetized with sodium pentobarbitone given i.v. The left femoral vein was cannulated for drug administration, and the left femoral artery was cannulated for recording arterial blood pressure. The right jugular vein was cannulated for recording pulmonary artery and wedge pressures.

Lead II ECG leads were used to record and derive the following hemodynamic parameters: systolic, diastolic, and mean blood pressure; systolic, diastolic, and mean pulmonary artery pressure, pulmonary capillary wedge pressure, and heart rate.

During the controls periods, inspired oxygen levels were 40%. To produce hypoxia, nitrogen was added to the inspired air to reduce inspired oxygen to 10%. Hypoxic conditions were maintained for 15 min periods, then returned to normoxic conditions (40% oxygen) for 30 min. Drug (batch #6642/00815) was given i.v. as a 2 min loading dose 15 min before the second hypoxic challenge, followed by a maintenance infusion that lasted through the second hypoxic challenge and subsequent normoxic period until the next dose was given. Drug was given to 6 dogs, and vehicle (0.038M sodium acetate buffer) was given to 4 dogs. The experimental procedure is summarized below (from Applicant's submission):



Drug was given at the following two dose ranges: (1) lower dose range (0.75-15 $\mu\text{g}/\text{kg}/\text{min}$ loading infusion followed by 0.05-1.5 $\mu\text{g}/\text{kg}/\text{min}$ maintenance infusion), and (2) higher dose range (15-270 $\mu\text{g}/\text{kg}/\text{min}$ loading infusion followed by 1-27 $\mu\text{g}/\text{kg}/\text{min}$ maintenance infusion) according to the following protocol (from Applicant's submission):

UK-92,480-27 was given at the following doses to 4 dogs:

<u>Infusion number</u>	<u>Loading infusion</u>	<u>maintenance infusion</u>
1	0.75 $\mu\text{g}/\text{kg}/\text{min}$	0.05 $\mu\text{g}/\text{kg}/\text{min}$
2	1.5 $\mu\text{g}/\text{kg}/\text{min}$	0.15 $\mu\text{g}/\text{kg}/\text{min}$
3	5.25 $\mu\text{g}/\text{kg}/\text{min}$	0.5 $\mu\text{g}/\text{kg}/\text{min}$
4	15 $\mu\text{g}/\text{kg}/\text{min}$	1.5 $\mu\text{g}/\text{kg}/\text{min}$

and at the following doses to 2 dogs:

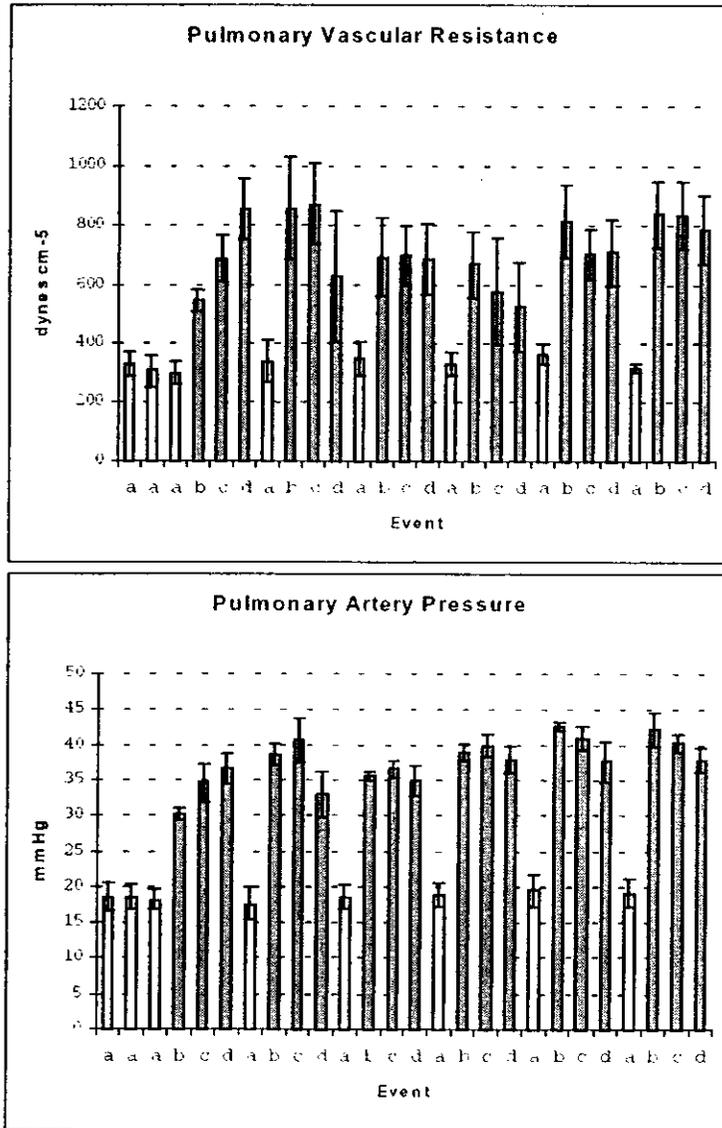
<u>Infusion number</u>	<u>Loading infusion</u>	<u>maintenance infusion</u>
1	15 $\mu\text{g}/\text{kg}/\text{min}$	1 $\mu\text{g}/\text{kg}/\text{min}$
2	30 $\mu\text{g}/\text{kg}/\text{min}$	3 $\mu\text{g}/\text{kg}/\text{min}$
3	90 $\mu\text{g}/\text{kg}/\text{min}$	9 $\mu\text{g}/\text{kg}/\text{min}$
4	270 $\mu\text{g}/\text{kg}/\text{min}$	27 $\mu\text{g}/\text{kg}/\text{min}$

Results: The effects of vehicle on pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP) are shown in Figure 1 (from Applicant's Figure B). As shown, hypoxia produced a consistent increase in both parameters through several rounds of vehicle administration, but there was variability in the responses.

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Figure 1 (from Applicant's Figure B)

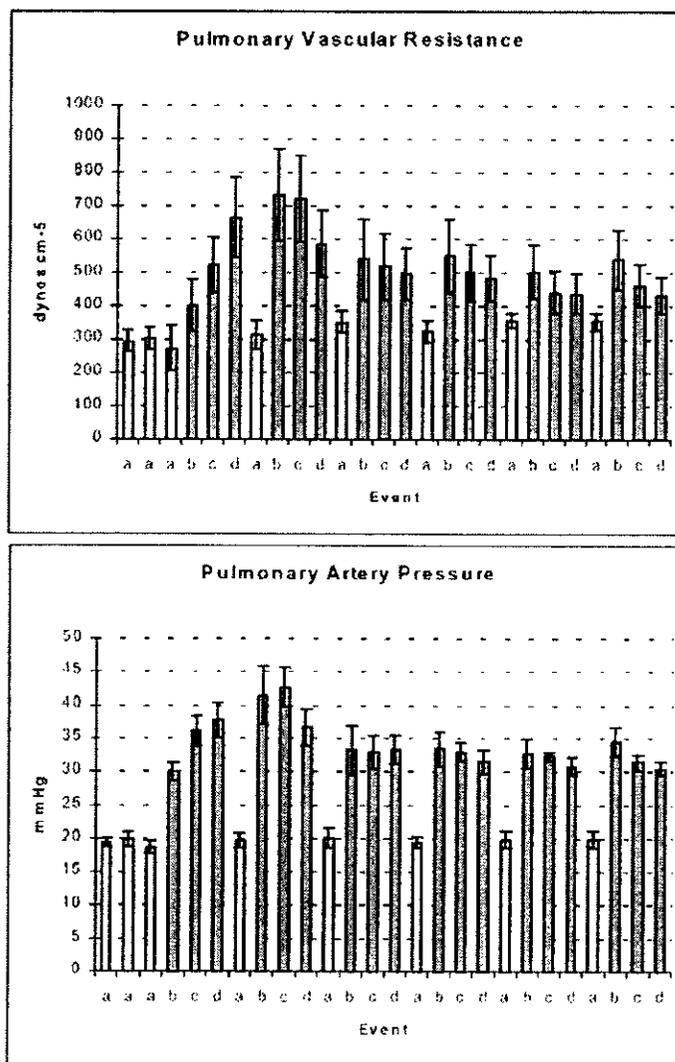
Figure B The effects of vehicle on haemodynamic parameters in a pentobarbitone anaesthetised dog model of hypoxic pulmonary vasoconstriction. Values are the mean \pm sem from 3 dogs. Normoxic values are designated 'a' and hypoxic values 'b,c and d' corresponding to 5, 10 and 15 minute time points respectively. Vehicle was administered following the second 15 minute hypoxic challenge.



The effects of drug at the lower dose range (0.75-15 $\mu\text{g}/\text{kg}/\text{min}$ loading infusion followed by 0.05-1.5 $\mu\text{g}/\text{kg}/\text{min}$ maintenance infusion) on pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP) are shown in Figure 2 (from Applicant's Figure A). As shown, drug administration reduced the increase in both parameters seen after hypoxia treatment, but increasing doses did not reduce the parameters further.

Figure 2 (from Applicant's Figure A)

Figure A The effects of UK-92,480-27 (lower dose group) on haemodynamic parameters in a pentobarbitone anaesthetised dog model of hypoxic pulmonary vasoconstriction. Values are the mean \pm sem from 4 dogs. Normoxic values are designated 'a' and hypoxic values 'b,c and d' corresponding to 5, 10 and 15 minute time points respectively. UK-92,480-27 was administered in increasing doses following the second 15 minute hypoxic challenge.

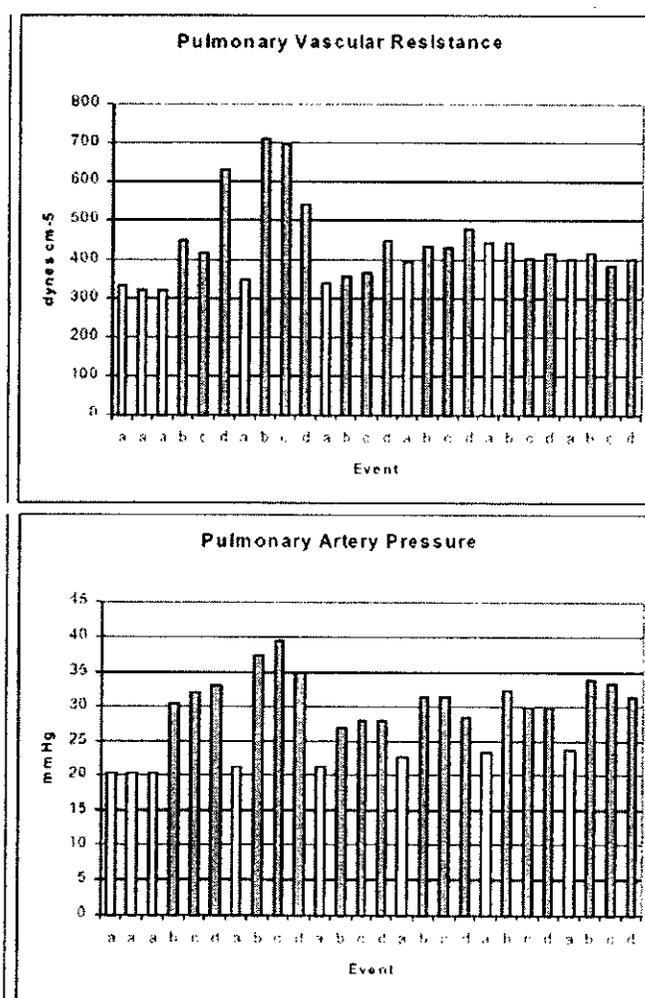


The effects of drug at the higher dose range (15-270 $\mu\text{g}/\text{kg}/\text{min}$ loading infusion followed by 1-27 $\mu\text{g}/\text{kg}/\text{min}$ maintenance infusion) on pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP) are shown in Figure 3 (from Applicant's Figure C). As shown, drug administration at higher doses reduced the increase in both parameters seen after hypoxia treatment, but increasing doses did not reduce the parameters further. In fact, pulmonary artery

pressure appeared to increase with increasing dosage. This result could be a reflection of inherent variability in the system.

Figure 3 (from Applicant's Figure C)

Figure C The effects of UK-92,480-27 (higher dose group) on haemodynamic parameters in a pentobarbitone anaesthetised dog model of hypoxic pulmonary vasoconstriction. Values are the mean from 2 dogs. Normoxic values are designated 'a' and hypoxic values 'b,c and d' corresponding to 5, 10 and 15 minute time points respectively. UK-92,480-27 was administered in increasing doses following the second 15 minute hypoxic challenge.



Other systemic hemodynamic parameters that were measured (e.g., systemic vascular resistance and mean blood pressure) were somewhat reduced by drug treatment as well when compared to predose hypoxic values, but generally to a lesser extent, when expressed as percent change, than that seen for the pulmonary parameters measured. However, there was marked variability in the test results and a clear dose-related effect could not be established (Figure 4;

Applicant's Figure 3), even though free plasma drug levels increased with increasing dosage (Table 1; Applicant's Table D).

Figure 4 (Applicant's Figure 3)

Figure 3 The effects of increasing doses (higher dose range) of UK-92,480-27 (n=2) on the hypoxia-induced increases in PAP and PVR and changes in mean arterial blood pressure and SVR during normoxia in pentobarbitone anaesthetised dogs. Values are the mean % change from predose.

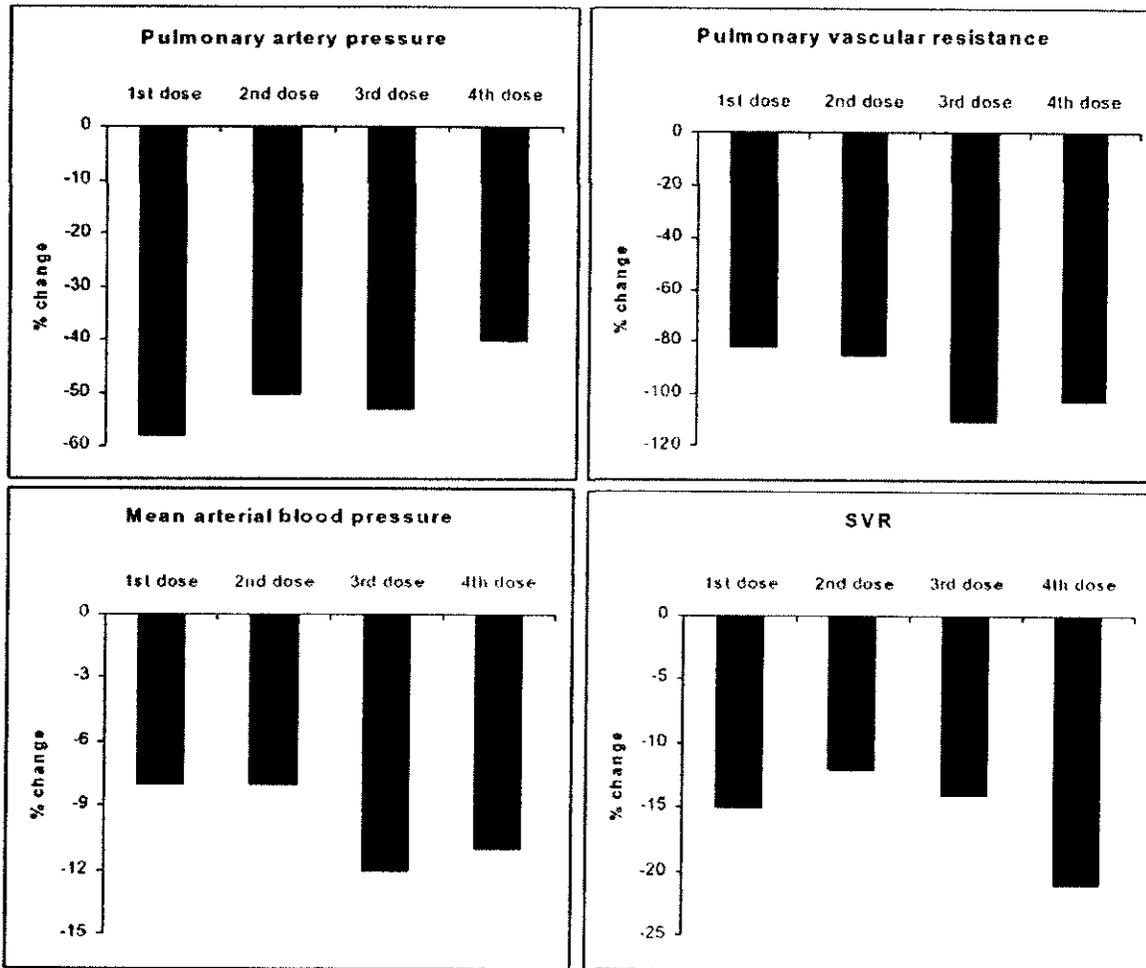


Table 1 (Applicant's Table D)

TABLE D Free plasma levels of UK-92480-27 (nM) following intravenous administration.

Lower dose group

	Expt 1	Expt 2	Expt 3	Expt 4	mean
Dose 1					BLQ
Dose 2					4.8
Dose 3					9.1
Dose 4					36.9

Higher dose group

	Expt 1	Expt 2	mean
Dose 1			15.7
Dose 2			57.8
Dose 3			170.7
Dose 4			462.6

Conclusions: Results in this dog model of pulmonary vasoconstriction showed that the intended effects of intravenously-administered sildenafil on reducing the hypoxia-induced increases in pulmonary artery pressure and pulmonary vascular resistance were greater than its unintended effects on decreasing systemic hemodynamic parameters (e.g., mean arterial blood pressure and systemic vascular resistance). These results would support the premise that sildenafil might have clinical utility to treat pulmonary hypertension in humans. However, there was marked variability in the responses seen across animals and a clear dose-related effect was not demonstrated, even though plasma drug levels increased with increasing dosage.

2.2.1.2. Inhibition of the Novel Human Recombinant Cyclic Nucleotide Phosphodiesterase (PDE) Enzymes 7 to 11 by Sildenafil, UK-103,320, UK-114,542, UK-150,564, UK-343,664 and UK-347,334

Purpose: Previous studies submitted in support of NDA #20-895 (Viagra; 1998) showed that 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). Since that time, additional PDE isozymes 7-11 have been identified. The present study was designed to examine possible inhibitory activity of newer PDE isozymes by sildenafil, two of its metabolites (UK-103,320 and UK-150,564), as well as that of other newer PDE5-selective inhibitors.

Methods: Human recombinant PDE isozymes 7-11 were purified by various methods. Enzyme activity was assayed by measuring the rate of conversion of radiolabeled cGMP to GMP or cAMP to AMP according to the isozyme's substrate specificity (Table 2; Applicant's Table 1).

Table 2 (Applicant's Table 1)

Table 1: Tissue mRNA distribution and cyclic nucleotide specificity of human PDE's 7-11⁽¹⁻⁶⁾.

Isozyme family	Distribution *	Substrate specificity
PDE7	abundant in skeletal muscle; also detectable in the heart and lymphocytes	cAMP specific
PDE8	widely distributed; highest levels found in testis, ovaries, small intestine and colon.	cAMP specific
PDE9	widely distributed; most abundant in spleen, small intestine and brain	cGMP specific
PDE10	abundant in putamen and caudate nucleus; also found in testis, and thyroid.	CAMP/cGMP
PDE11	abundant in skeletal, cardiac and smooth muscle cells, inc. those of the prostate, penis & bladder; also in secretory cells, testis, liver and kidney	cAMP/cGMP

* Based on mRNA

Inhibition studies to determine the IC₅₀ were conducted in the presence or absence of inhibitor (i.e., sildenafil, its metabolites, and other PDE5 inhibitors).

Results: IC₅₀ values for sildenafil and its two metabolites (UK-103,320 and UK-150,564) for human recombinant PDE's 7-11 and human corpus cavernosum PDE5 (historical data) are shown in Table 3 (Applicant's Table 3). Sildenafil and its metabolites showed selective (>100-fold) specificity toward PDE5 (lower IC₅₀) when compared to PDEs 7-11.

Table 3 (Applicant's Table 3)

Table 3: IC₅₀ values for inhibition of human recombinant PDE's 7-11 and human corpus cavernosum PDE5 by sildenafil, UK-103,320 and UK-150,564 (data represent geometric mean with 95% confidence interval in parentheses; PDE's 7-11: n=3; PDE5: n=2-15).

	IC ₅₀ (µM)					
	PDE5	PDE7	PDE8	PDE9	PDE10	PDE11
Sildenafil	0.0035	21.3 (16.5-27.4)	29.8 (17.0-52.5)	2.61 (1.39-4.91)	9.80 (6.30-15.3)	2.73 (2.46-3.04)
UK-103,320	0.0089	>10	15.1 (5.40-41.7)	5.23 (4.30-6.35)	16.5 (13.3-20.4)	1.43 (0.760-2.67)
UK-150,564	0.0377	>100	>10	21.8 (12.3-38.9)	45.0 (44.0-46.0)	14.2 (10.6-19.0)

* historical data^(b)

Conclusions: Sildenafil and its active metabolites showed selective specificity toward PDE5 when compared to all other PDEs identified to date. The PDE with the lowest IC₅₀, other than PDE5, was PDE6 (10-fold selectivity of sildenafil for PDE5 when compared to PDE6). Of the PDEs examined in this study, the PDE's with the lowest IC₅₀ were PDE9 (IC₅₀ = 2.61 µM) and PDE11 (IC₅₀ = 2.73 µM). This represents a >700-fold selectivity of sildenafil for PDE5 when compared to PDE's 9 and 11. These results suggest that sildenafil at therapeutic concentrations will have minimal effect on the activity of these enzymes in the tissues in which they are expressed (see Table 2; Applicant's Table 1).

2.2.2. Secondary Pharmacodynamics

2.2.2.1. Determination of cGMP-hydrolyzing PDE Isozyme Activity in Human Cardiac Muscle

Purpose: Previous studies have suggested that cGMP levels may be involved in modulating cardiac myocyte contractility. There are theoretical concerns that elevated levels of cGMP, resulting from inhibition of PDE5-mediated breakdown of cGMP by sildenafil, can lead to inhibition (or depletion) of PDE3 which can lead to elevation of cAMP and concomitant inotropic effects. To address these concerns, this study examined cGMP-hydrolyzing PDE5 enzyme activity in human cardiac tissue.

Methods: Frozen human atrium and ventricle tissues were obtained. Tissue samples were extracted and soluble phosphodiesterase fractions isolated by

Fractions containing phosphodiesterase activity were assayed in inhibition studies by measuring the rate of conversion of radiolabeled cGMP to GMP in the presence or absence of a PDE1-selective inhibitor (UK-90,234) and two PDE5-selective inhibitors (UK-92,480 or sildenafil; and UK-343,664). Also, Western blotting was performed to determine protein expression of PDE5 in human cardiac tissue.

Results: PDE1 and PDE5 activity co-eluted in a single major peak (Peak 1) (Figure 5; Applicant's Figure 1). The latter peaks correspond to PDE2 and PDE3, respectively.

Figure 5 (Applicant's Figure 1)

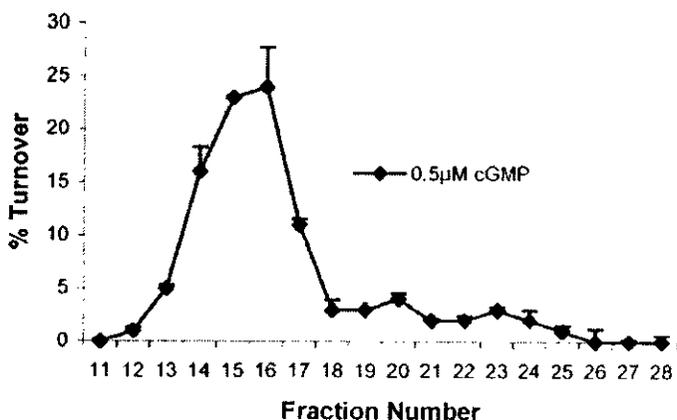


Figure 1. Typical profile of phosphodiesterase (PDE) activity in human atrium and ventricle from donor sample 2

When assayed in the presence or absence of selective PDE1 and PDE5 inhibitors, PDE activity in Peak 1 from a representative donor sample was shown to be reduced by only the PDE1-selective inhibitor (UK-90,234), and not by the two PDE5-selective inhibitors (UK-92,480 or sildenafil; or UK-343,664) (Figure 6; Applicant's Figure 4).

Figure 6 (Applicant's Figure 4)

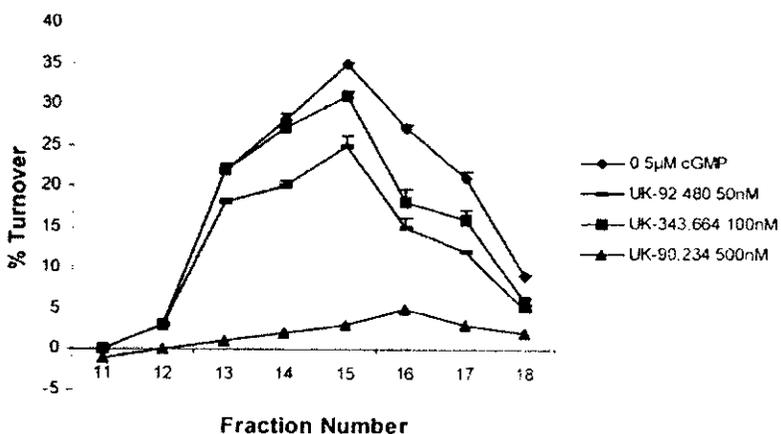


Figure 4. Typical PDE activity profile from donor sample 3 (LA3, LV3) in presence of PDE5 and PDE1 inhibitors.

Western blot analysis of human heart samples using an antiserum capable of detecting all known human PDE5 isoforms showed undetectable or weak immunoreactivity for PDE5 (data not shown).

Conclusions: Analysis of human heart atrium and ventricle samples showed that PDE1 was the major PDE isozyme found in the human heart, followed by PDE2 and PDE3. PDE5 activity was present, but in much lower amounts. These results are consistent with published data. Therefore, therapeutic use of a selective PDE5 inhibitor, such as sildenafil, is expected to have minimal effects on modulating cAMP-mediated contractility of the heart.

2.2.3. Safety Pharmacology

2.2.3.1. To Determine Whether Sildenafil (UK-92,480-10) Has Agonist/Antagonist Activity at Human A2a Receptors Expressed in HEK Cells

Purpose: Previous binding studies using isolated membrane fractions have shown that sildenafil binds to human adenosine A2a receptors ($IC_{50} = 1 \times 10^{-7}$). Since adenosine A2a receptors are expressed on coronary arteries, and activation of these receptors leads to coronary vasodilation, it is not clear if this binding activity by sildenafil for A2a receptors can have functional consequences. Therefore, this study examined whether sildenafil possesses any agonist (or antagonist) activity for human adenosine A2a receptors expressed in human embryonic kidney (HEK) cells using intracellular cAMP levels as a measure of A2a receptor activation.

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Methods: Human embryonic kidney (HEK) cells expressing human A2a receptors were treated with various concentrations of either sildenafil or CGS-21680, an A2a agonist, and intracellular cAMP levels measured.

Results: Results showed that treatment with sildenafil did not increase intracellular cAMP levels at concentrations up to 3000 nM (3×10^{-6} M), whereas treatment with the A2a agonist CGS-21680 produced a dose-dependent increase in cAMP levels (Figure 7; Applicant's Figure 1).

Figure 7 (Applicant's Figure 1)

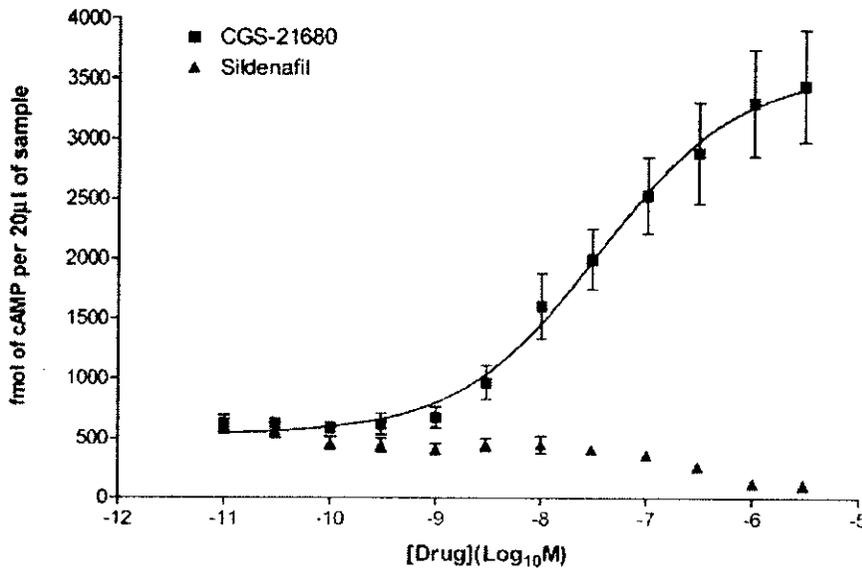


Figure 1. Effect of CGS-21680 and Sildenafil on cAMP levels in A2a HEK cells. (n=4).

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Treatment of HEK cells with the A2a agonist CGS-21680 in the presence of increasing concentrations of sildenafil (30-3000 nM) showed that sildenafil acted as a competitive antagonist by shifting the curve to the right, an indication that more of the cAMP agonist was required to produce an equivalent increase in cAMP (Figure 8; Applicant's Figure 2). These results suggest that sildenafil possesses some antagonist activity for adenosine A2a receptors ($pK_B = 6.80$), results inconsistent with the membrane binding studies previously conducted.

Figure 8 (Applicant's Figure 2)

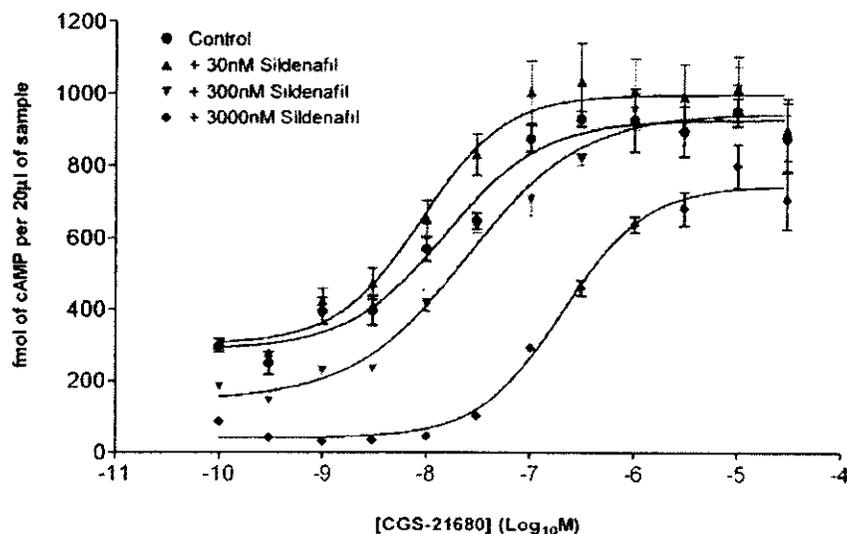


Figure 2. Effect Of Increasing Sildenafil Concentrations on CGS-21680-Induced cAMP Accumulation In HEK Cells. Only 3×10^{-8} , 3×10^{-7} and 3×10^{-6} data sets are shown for clarity (n=4).

Conclusions: Sildenafil at concentrations up to 3000 nM to was shown not to possess agonist activity for adenosine A2a receptors when tested directly in A2a receptor-expressing HEK cells. However, it was shown to possess antagonist activity at the A2a receptor when tested in the presence of an adenosine A2a receptor agonist. These results suggest that sildenafil is unlikely to produce additional coronary vasodilation through A2a-receptor-mediated increases in intracellular cAMP levels.

2.2.3.2. An In Vitro Evaluation of the Effect of Sildenafil (UK-92,480) on Isoprenaline-induced Contractility in Rabbit Isolated Papillary Muscle

Purpose: A relatively recent study (Stieff *et al.*, 2000) reported that sildenafil at concentrations of 10^{-7} - 10^{-5} M raises cAMP in the heart, and may possess positive inotropic activity, an effect that could lead to adverse cardiovascular side effects. This study examined the effect of sildenafil on isoprenaline-induced contractility in the rabbit isolated papillary muscle.

Methods: Rabbit papillary muscles were prepared, suspended in buffer in a jacketed organ bath, and attached to isometric strain gauges. Tissues were incubated in increasing concentrations of isoprenaline until a maximum response was observed to establish a control curve.

After equilibration, tissues were incubated with increasing concentrations of isoprenaline in the presence of either sildenafil, amrinone (PDE3 inhibitor used as a positive control), or vehicle (negative control). A study to examine the concentration-response curve for sildenafil in the absence of isoprenaline was performed to determine if sildenafil alone possessed any effect on contractility. Results are expressed as the percentage of the isoprenaline-induced maximum increase.

Results: Pretreatment of rabbit papillary muscle with sildenafil at concentrations up to 10^{-6} M did not affect the contractile response to isoprenaline, whereas pretreatment with the positive control amrinone (10^{-5} M) did increase isoprenaline-induced contractility (Figure 9; Applicant's Figure 3). However, pretreatment of papillary muscle with sildenafil at 10^{-5} M did show an enhanced contractile response to isoprenaline (Figure 10; Applicant's Figure 4). Treatment with sildenafil alone at concentrations up to 10^{-5} M did not show any positive inotropic effects on rabbit papillary muscle (data not shown).

Figure 9 (Applicant's Figure 3)

Results Expressed as Percentage of the Isoprenaline-Induced Maximum Increase

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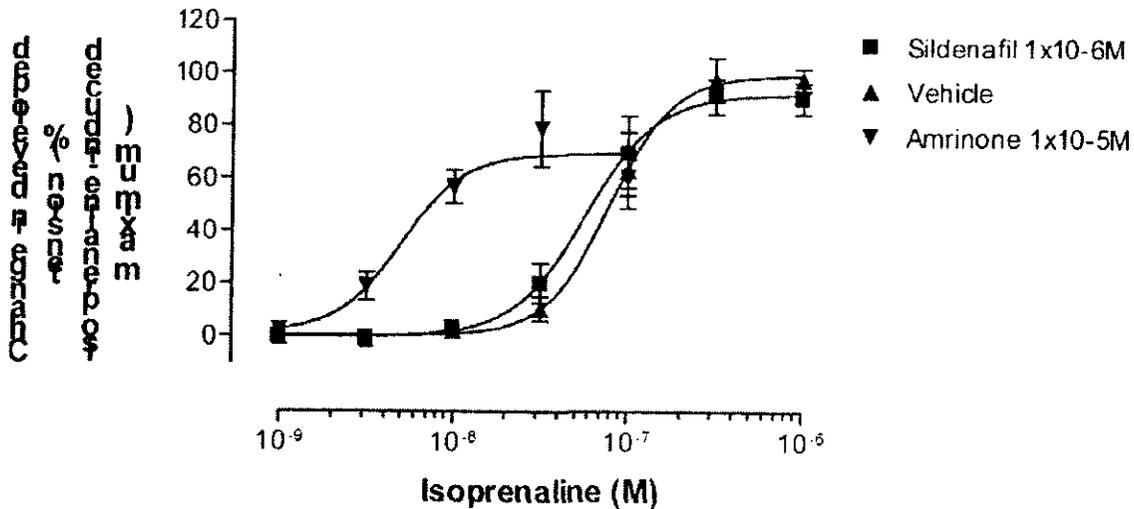


Figure 3: The contractile response to isoprenaline was not significantly affected by sildenafil (1×10^{-6} M) pre-treatment compared to time-matched vehicle. Amrinone (1×10^{-5} M) pre-treatment, did significantly ($p < 0.001$) enhance isoprenaline induced contractility in the isolated papillary muscle compared to the time-matched vehicle group. A total of between 6-8 tissues per treatment group were studied, with each point on this graph representing a mean isoprenaline response from 3-8 tissues in each treatment group.

Figure 10 (Applicant's Figure 4)

Results Expressed as Percentage of the Isoprenaline-Induced Maximum Increase

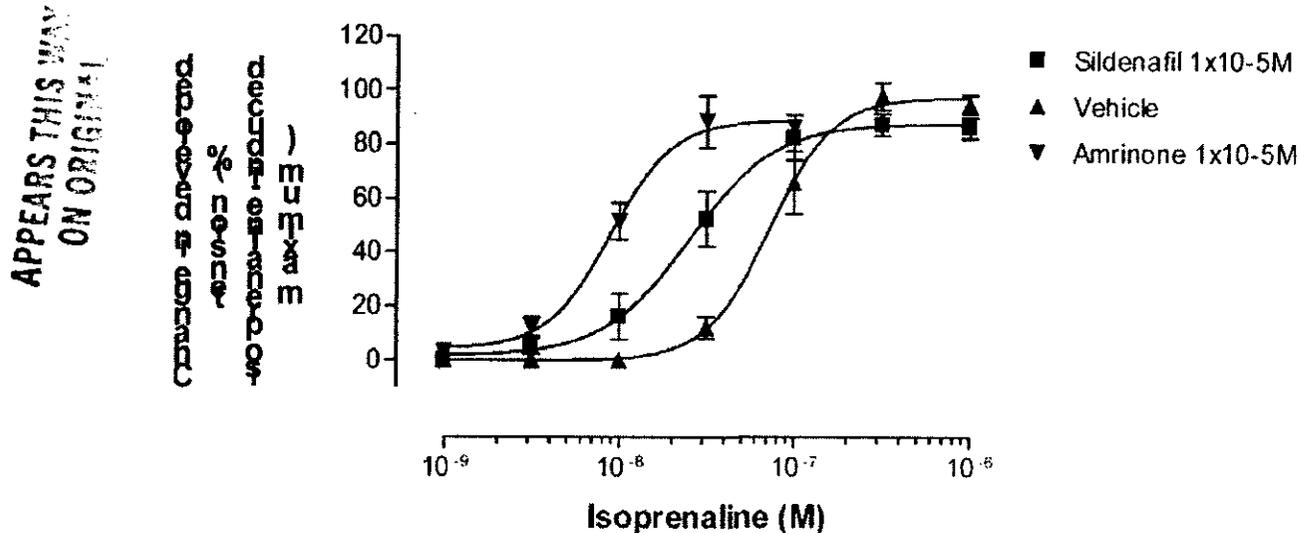


Figure 4: Pre-treatment with sildenafil ($1 \times 10^{-5} \text{M}$) did significantly ($p < 0.05$) enhance the contractile response to isoprenaline compared to time-matched vehicle. Time-matched amrinone ($1 \times 10^{-5} \text{M}$) pre-treatment, significantly ($p < 0.001$) potentiated isoprenaline-induced contractility compared to vehicle as expected. A total of between 6-8 tissues per treatment group were studied, with each point on this graph representing a mean isoprenaline response from 4-8 tissues in each treatment group, apart from the isoprenaline $1 \times 10^{-7} \text{M}$ response, in the amrinone treatment group which represents the response from 1 tissue in this group.

Conclusions: Treatment of isolated rabbit papillary muscle with sildenafil at 10^{-5}M was shown to shift the isoprenaline-response curve to the left, indicating a positive inotropic effect in this test system. However, according to the Applicant, 10^{-5}M ($= 10,000 \text{ nM}$) is 250X higher than the free plasma concentration (40 nM) achieved in man after a 100 mg therapeutic dose of sildenafil. Although the concentrations of sildenafil required to produce a positive inotropic effect in this test system appear to far exceed those achieved during clinical use, extrapolation of drug concentrations used *ex vivo* to those seen *in vivo* should be used with caution.

2.3. PHARMACOKINETICS

2.3.1. Distribution

2.3.1.1. Determination of Brain Penetration of [¹⁴C]-Sildenafil in Male Rat Following Single Subcutaneous (2 mg/kg) Administration

Concentrations of radioactivity were determined in plasma, brain and CSF of male rats for up to 1h following subcutaneous administration of [¹⁴C]-sildenafil citrate. Throughout the

time course of the experiment the levels of total radioactivity in both brain tissue and CSF were lower than those in plasma. The concentrations relative to plasma were in the range 12-27% in brain and 1-3% in CSF.

These results showed that sildenafil and/or its metabolites penetrate the brain in the rat after subcutaneous administration.

2.4. OVERALL SUMMARY AND RECOMMENDATIONS

This NDA proposes to use an already marketed drug (sildenafil citrate) for a new indication (PAH). Several primary and secondary pharmacodynamic studies, pharmacokinetic studies, and safety pharmacology studies were submitted to support the new indication. No toxicology studies were submitted, since these have already been submitted for NDA #20-895 (Viagra; treatment of erectile dysfunction), and have been cross-referenced in the current NDA.

Sildenafil's mechanism of action is through inhibition of the breakdown of cyclic guanosine monophosphate (cGMP) by phosphodiesterase 5 (PDE5). Activation of the enzyme guanylate cyclase by nitric oxide (NO) and prolongation of cGMP levels by inhibition of its breakdown by sildenafil result in prolonged vascular smooth muscle relaxation and vasodilatation. Given that NO and cGMP are involved in modulating pulmonary vascular tone, and that high levels of PDE5 are found in pulmonary epithelium, the use of sildenafil to increase NO/cGMP-dependent pulmonary vasodilatation as a treatment for PAH was studied.

2.4.1. Pharmacology

Sildenafil was studied in an animal model of pulmonary hypertension. Pulmonary vasoconstriction was produced in beagle dogs by hypoxia (10% oxygen). Sildenafil was then administered to the dogs by i.v. infusion and effects on pulmonary hemodynamics examined. Results showed that the intended effects of intravenously-administered sildenafil on reducing the hypoxia-induced increases in pulmonary artery pressure and pulmonary vascular resistance were greater than its unintended effects on decreasing systemic hemodynamic parameters (e.g., mean arterial blood pressure and systemic vascular resistance). However, there was marked variability in the responses seen across animals and a clear dose-related effect was not demonstrated, even though plasma drug levels increased with increasing dosage.

Since the approval of sildenafil (Viagra) in 1998 for the treatment of erectile dysfunction, several new PDE isozymes (PDEs 7-11) have been identified. Possible inhibitory effects of sildenafil on these newer PDEs were examined. Results showed that sildenafil and its active metabolites showed selective specificity toward PDE5 when compared to all other PDE's identified to date. The PDE with the lowest IC_{50} , other than PDE5, was still PDE6, a PDE isozyme found in the retina (10-fold selectivity of sildenafil for PDE5 when compared to PDE6).

Previous studies have suggested that cGMP levels may be involved in modulating cardiac myocyte contractility through a cAMP-mediated mechanism. There are theoretical concerns that elevated levels of cGMP, resulting from inhibition of PDE5-mediated breakdown of cGMP by sildenafil, can lead to inhibition (or depletion) of PDE3 which can lead to elevation of cAMP and inotropic effects. To address these concerns, human cardiac tissue was examined for presence of cGMP-hydrolyzing PDE5 enzyme activity. Results showed that PDE1 was the major PDE isozyme found in the human heart, followed by PDE2 and PDE3. PDE5 activity was present, but in much lower amounts. Therefore, therapeutic use of a selective PDE5 inhibitor, such as

sildenafil, is expected to have minimal effects on modulating cAMP-mediated contractility of the heart.

To further explore the possibility that sildenafil raises cAMP levels in the heart, an effect that could lead to adverse cardiovascular side effects, the effects of sildenafil on isoprenaline-induced contractility in the rabbit isolated papillary muscle was studied. Results showed that treatment of isolated rabbit papillary muscle with sildenafil shifted the isoprenaline-response curve to the left, indicating a positive inotropic effect. Although the concentrations of sildenafil required to produce a positive inotropic effect in this test system appear to far exceed (250X) those achieved during clinical use, extrapolation of drug concentrations used *ex vivo* to those seen *in vivo* should be used with caution.

Previous binding studies using isolated membrane fractions have shown that sildenafil binds to human adenosine A2a receptors. Since adenosine A2a receptors are expressed on coronary arteries, and activation of these receptors leads to coronary vasodilation, sildenafil was studied to determine if it possessed any agonist or antagonist activity for human adenosine A2a receptors. Results showed that sildenafil did not possess agonist activity for adenosine A2a receptors when tested directly in A2a receptor-expressing HEK cells. However, it was shown to possess antagonist activity at the A2a receptor when tested in the presence of an adenosine A2a receptor agonist. These results suggest the sildenafil is unlikely to produce additional coronary vasodilation through A2a-receptor-mediated increases in intracellular cAMP levels.

Sildenafil was found to penetrate the brain and CSF of rats following subcutaneous administration. The concentrations relative to plasma were in the range 12-27% in brain and 1-3% in CSF.

2.4.2. Toxicology

No toxicology studies were submitted. *In vitro* and *in vivo* toxicity studies supporting the chronic administration of sildenafil were previously submitted and reviewed under NDA #20-895 (Viagra; sildenafil citrate; Pfizer, Inc.). In those studies conducted over relatively large dose ranges, no adverse cardiovascular (including proarrhythmogenic and hemorrhagic), autonomic, cytotoxic, tumorigenic/genotoxic, or reproductive toxicities were found.

2.4.3. Labeling

Due to the differences between the dose used for erectile dysfunction (100 mg Viagra) and the 20 mg three times a day (t.i.d.) dosing recommended for pulmonary hypertension (60 mg/day Revatio), the Revatio draft labeling sections for (1) Carcinogenesis, Mutagenesis, Impairment of Fertility and (2) Pregnancy differ from the labeling for Viagra, specifically the dose-exposure multiples for animals relative to humans. The Applicant was asked to provide the basis for the animal to human multiples given. The Applicant responded as follows, via an amendment to the NDA (dated 4/27/05).

Carcinogenesis, Mutagenesis, Impairment of Fertility

The dose-exposure multiples in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section were not correct due to a calculation error, in that daily animal exposures were

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 § 552(b)(4) Trade Secret / Confidential

 § 552(b)(5) Deliberative Process

 § 552(b)(5) Draft Labeling

The revised labeling provided by the Applicant is as follows:

*"Sildenafil was not carcinogenic when administered to rats for 24 months
... , respectively, the exposure in
Recommended Human Dose (RHD) of 20 mg t.i.d."*

Pregnancy

The Applicant's proposed labeling for this section is as follows:

Pregnancy

Pregnancy Category B. No evidence of teratogenicity, embryotoxicity or fetotoxicity was observed in rats and rabbits, which received up to 200 mg/kg/day during organogenesis: 32
and 68 times the RHD

There are no adequate and well-controlled studies of sildenafil
in pregnant women.

2.4.4. Recommendations

Based on: (1) the extensive clinical experience with sildenafil for the treatment of erectile dysfunction at doses comparable to those proposed for the new indication, (2) the extensive pharmacology and toxicology studies conducted for both indications (i.e., erectile dysfunction and pulmonary arterial hypertension), and (3) the lack of any significant safety concerns for the indicated patient population at the recommended dosing regimen of 20 mg t.i.d. (3X/day), NDA #21-845 is approvable from a pharmacology and toxicology perspective.

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Thomas Papoian
5/12/05 10:42:15 AM
PHARMACOLOGIST

Chuck, you have signed-off on this review. Tom

Charles Resnick
5/20/05 05:17:04 PM
PHARMACOLOGIST