Review Article

Sildenafil (Viagra®): A Pharmacokinetic (PK) Review

ABSTRACT

Introduction: Erectile Dysfunction (ED) is the inability to achieve or/and maintain a sufficient penile erection for successful, satisfactory, and pleasant vaginal intercourse. National Institute of Health's division of NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) defines erectile dysfunction as a condition in which one person cannot get or keep an erection firm enough for satisfactory sexual intercourse. The usual symptoms include problems achieving a solid erection, difficulties maintaining the erected condition of the penis, and decreased sexual desire. Several factors can contribute to these penile disorders. These include neurogenic injury, endocrinological disorders, drug-affected pathology, cardiovascular disease, etc.

Methods: A careful literature search was conducted to compile all the recent information available for sildenafil PK. Primary literature searches included PubMed, Google Scholar, Wiley online resources, etc.

Results: After the completion of the lengthy process of drug discovery, sildenafil citrate was approved by the FDA to be used for erectile dysfunction in March 1998. It was the very first oral treatment for erectile dysfunction.

Conclusion: Although several selective PDE 5 inhibitors are available now, sildenafil citrate is still one of the most prescribed drugs for treating penile erectile disorders.

Keywords: Erectile dysfunction, sex, Viagra®, intercourse, PDE5i

1. INTRODUCTION

Erectile Dysfunction (E.D.) is the inability to achieve or/and maintain a sufficient penile erection for successful, satisfactory, and pleasant vaginal intercourse [1-3]. National Institute of Health's division of NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) defines erectile dysfunction as a condition in which one person cannot get or keep an erection firm enough for satisfactory sexual intercourse'[4, 5]. The usual symptoms include problems achieving a solid erection, difficulties maintaining the erected condition of the penis, and decreased sexual desire. Several factors can contribute to these penile disorders, like neurogenic injury, endocrinological disorders, drug-affected pathology, cardiovascular disease, etc. [1-5].

The prevalence of E.D. is highly dependent on the age of the male population. Several studies have estimated that less than 10% of men who are younger than 40 years of age are affected by E.D. The prevalence range is between 2 to 9% within the age range of 40-49 years. However, it is increased to 40% if the age range is 60-69%. The highest prevalence of E.D. was 50-100% in men over 70 years [3]. A similar study in the U.S. showed that the bulk of E.D. affects about 18 million men in America of the age group of 20 years older. The prevalence was more than 70% in men in the U.S. aged 70 years or older. The same study found that the majority was highly significant in men with cardiovascular conditions. Other factors such as diabetes, lack of exercise, and lower sexual education exacerbate the situation [6].

About twenty years ago, men with impotence received redemption in the form of a little blue pill. That blue pill that treats impotence named sildenafil or Viagra (brand name, Pfizer inc.) was patented in 1996 and approved by the Food and Drug Administration (FDA) on March 27, 1998, [7]. Basically, sildenafil is a

potent, selective, reversible inhibitor of phosphodiesterase type 5 (PDE5) used to treat pulmonary arterial hypertension. But, its serendipitous discovery indicates the treatment of impotence and male erectile dysfunction, which ultimately opened a new era of clinical application for this class of drug [8]. The happenstance of its effects on penile erection provided a quantum leap in the treatment of erectile dysfunction (E.D.) [9]. Back In 1974, Zaprinast was synthesized and was later characterized as the first selective PDE 5 inhibitor. Eventually, studies revealed that Zaprinast was not selective only for PDE5. Later, Exploring PDE5 as a target for a range of cardiovascular disorders like hypertension and angina pectoris, Terrett et al. (1996) found that PDE5 was the predominant hydrolyzing enzyme in the cytosolic fraction found in the corpus cavernosum of humans and suggested that one of their synthesized inhibitors which were sildenafil [9]. Indisputably, at that time, study stances that it was a potent and highly selective PDE5 inhibitor and could be useful as an orally active treatment for male ED [10]. Now, the generic name Sildenafil is an oral tablet that can treat both conditions, but one brand-named drug can treat only one of the conditions. Viagra (brand name) treats E.D. when a man cannot get or maintain a penile erection. On the other hand, Revatio (brand name, Pfizer) is a citrate salt of sildenafil, used to treat PAH when the blood pressure in the lungs is too high. Besides that, due to the vasodilatory activity and high levels of PDE5 expression in the lung tissue, researchers rationally considered that the drug has some possible therapeutic effects against pulmonary fibrosis, a complication of the COVID-19 disease [8].

2. MECHANISM OF ACTION OF SILDENAFIL:

From ancient times, penile erection has been considered a remarkably interesting topic. So many antique hypotheses and experimental findings have been given by several types of scientists, philosophers, and investigators. Hippocrates (460-370 BC) thought that the erection of the penis is caused by a vital spirit flowing through the penis, which is based on the perfect balance of four humors and four body elements. The four humors were blood, phlegm, yellow bile, and black bile. The four elements were earth, air, fire, and water. He considered the testes as the pulleys connecting the penis. On the other hand, another great philosopher Aristotle (384-322BC), hypothesized that the penile erection is an imagination-driven movement of the penis. He considered the testes as the fulcrums helping to lift the penis. Later Galen (129-200) considered that the penile erection is caused by the accumulation of air in the corpus cavernosum. But Da Vinci was the first person who correctly theorized that blood causes the erection of the penis. And ultimately, Von Haller from Switzerland theorized that this penile erection is controlled by the nervous system [11]. These hypotheses demanded a deeper understanding of the penile erection, and animal studies were performed to understand the underlying mechanism behind this physiologic activity. It was found that the nervous system controlled smooth muscle relaxation in the corpora cavernosa and was responsible for the erection and hardening of the penis [11-15].

Figure 1 demonstrates the mechanism of penile erection. Several neurotransmitters are responsible for the erection of the penis. The most important neurotransmitter is Nitric Oxide (NO) [11-15]. The contraction and relaxation of penile smooth muscles determine the flaccidity and rigidity of the penis, respectively. Intracellular Ca^{2+} ion concentration is the determinant of cell contraction or muscle contraction. Therefore, if the Ca^{2+} concentration decreases, the contraction will be decreased, and the relaxation will be triggered, resulting in a rigid and erect penis. Due to various kinds of sexual arousal, non-adrenergic, non-cholinergic neurotransmitter NO is synthesized due to the action of NO synthases. NO is produced from the nerve ends as well as from the endothelial cells. Diffused into the smooth muscles, they bind with the guanylyl cyclase to activate cyclic guanosine monophosphate (cGMP) synthesis. Upon synthesis, cGMP triggers the cGMP-dependent ion channels and/or cGMP-dependent protein kinases. This result in the reduction of the intracellular Ca^{2+} ion concentration. So, contraction is reduced, and the relaxation is induced to result in penile erection [1, 3, 5, 12, 16, 17].

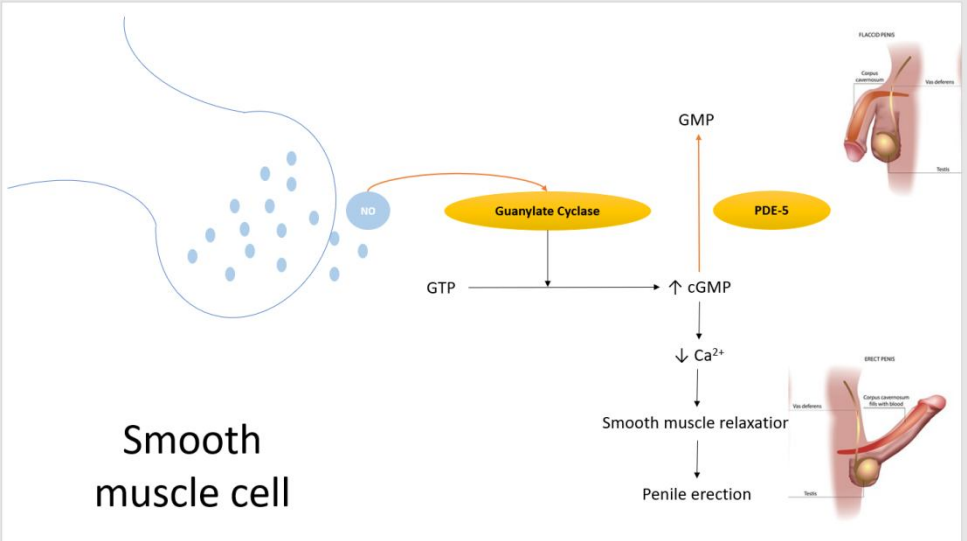


Figure 1: Physiology and mechanism of penile erection

Sildenafil citrate is used to treat erectile dysfunction (E.D.) in men. It inhibits the phosphodiesterase 5 (PDE5) enzyme selectively. PDE5 is the main enzyme to degrade cGMP in the penile smooth muscle. Sildenafil inhibits the degradation of cGMP by selectively blocking the enzyme PDE5. Nitric oxide (NO), when released from nerve endings or endothelial cells, it binds with guanylyl cyclase. Activated guanylyl cyclase increases the concentration of cGMP intracellularly. Intracellular cGMP causes a reduction in Ca2+ concentration to provide muscle relaxation and erection of the penis.

PDE5 is a negative feedback mechanism to degrade the cGMP and helps return the penis to its original flaccid condition. Sildenafil citrate blocks the catalytic site of PDE5. Because the molecular structures of cGMP and sildenafil citrate are similar. Sildenafil competes with the cGMP for the binding site of the PDE5 (Figure 2). As a result, it extends the time needed to degrade the cGMP in the penile smooth muscle and provides erection for a longer time [18-21].

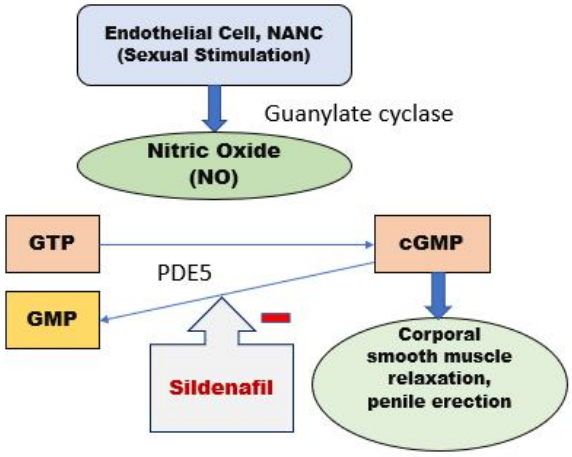


Figure 2: Sildenafil mechanism of action for penile erection

Besides that, there have been numerous reports of the use of sildenafil in cardiovascular diseases, the Raynaud phenomenon, cystic fibrosis, cancer, diabetes, and neurological disorders such as Alzheimer's. Sildenafil also has 10% activity against PDE6. PDE6 is a key enzyme in the phototransduction cascade in the retina. Recent ocular imaging developments have further revealed the influence of sildenafil on ocular hemodynamics, particularly choroidal perfusion [7]. Here, the photoisomerization process activates the rhodopsin. As a result, transducin activation is responsible for PDE6 activation. This PDE6 breaks the

cGMP, resulting in photoreceptor hyperpolarization and visual sensitivity by stopping Sodium and Calcium influx into outer segments [7, 22] [7]. Sildenafil inhibits the PDE6 so that further process cannot occur and visual activity remain clear.

As we know, PDE5 degrades cyclic guanosine monophosphate (cGMP), and with this upstream of cGMP, usually, the amino acid L-arginine is converted by three varieties of the enzyme nitric oxide synthase (NOS) into nitric oxide (NO). NO is a small cell-permeable gas molecule that diffuses across the plasma membrane, activating soluble guanylyl cyclase (sGC) and this sGC converts guanosine triphosphate (GTP) into cGMP. In A.D. patients, the activity of the NOS/NO/cGMP pathway is severely impaired, hence NOS activity is significantly decreased in A.D. patients. NOS/NO/cGMP has a multi mechanism of action thus signaling dysfunction is an important therapeutic target in A.D. and PDE5 inhibitors have a great impact on it [23, 24].

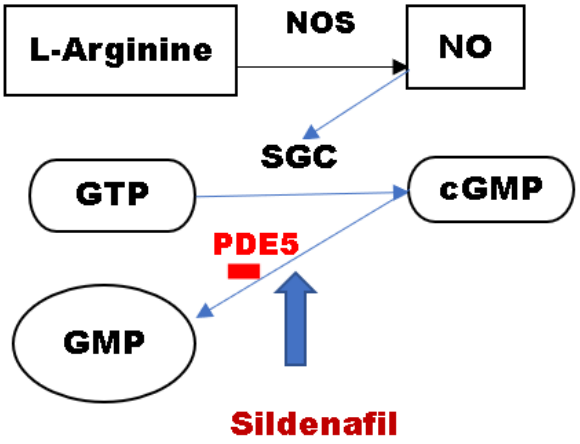


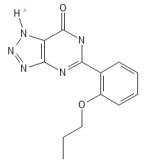
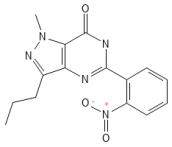
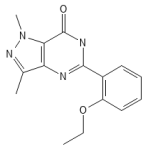
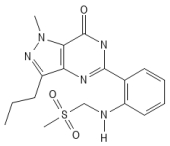
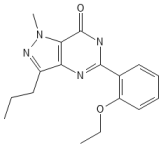
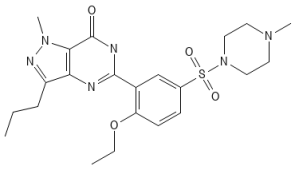
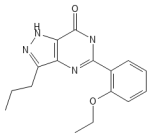
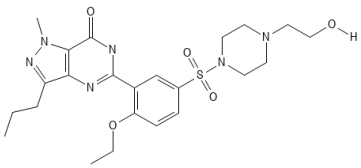
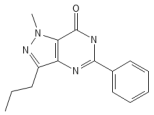
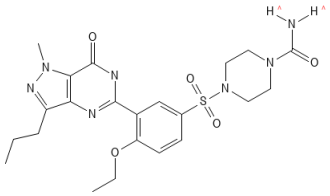
Figure 3: Sildenafil mechanism of action in A.D.

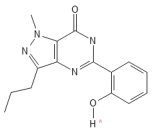
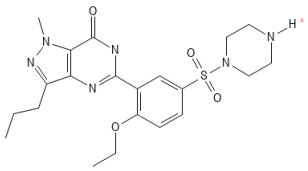
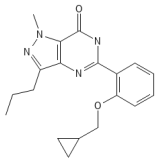
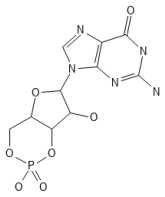
In diabetic nephropathy (D.N.) mechanisms of PDE5 inhibitors are yet to be explored. But in general, they have some effects on D.N. patients. In D.N. glomerular pressure increases due to alteration in the cyclic guanosine monophosphate (cGMP)-nitric oxide (NO) pathway. production of cGMP helps NO dilates the blood vessel of the glomeruli by stimulating the process. Increased cGMP starts to relax the renal vascular smooth muscle and PDE5 inhibitor breaks down cGMP Thereby it improves metabolic and hemodynamic pathophysiological factors like- inflammatory pathways, all of which are dysfunctional in D.N. Other studies have shown that sildenafil inhibits extracellular matrix accumulation partially by affecting the balance between matrix metalloproteinases and their inhibitors, thereby attenuating renal damages. Besides that, new research has shown that Viagra (the market name of sildenafil) increases insulin sensitivity in people with prediabetes [10].

Lately, it has been reported by several researchers that sildenafil has some anticancer efficacy by inhibiting of PDE5 by high-affinity inhibitors was suggested as a chemoprotective approach for colorectal cancer. A recent study stance that, giving orally sildenafil in mice shown significant suppression of cancer cells proliferation, especially in preliminary stages of carcinogenesis. Moreover, it has also Antitumor effect in colorectal cancer which has been reported for human cell lines in vitro and in vivo [8].

3. STRUCTURE ACTIVITY RELATIONSHIP (SAR) AND SILDENAFIL:

Table 1 represents all the compounds necessary to discuss the SAR related to chemical analogues related to sildenafil discovery.

a (Zaprinast)		h	
b		i	
c		j (Sildenafil)	
d		k	
e		l	

f		m	
g		cGMP	

The drug discovery process of sildenafil citrate, well known as Viagra, is a long story of 1980s. It was first produced chemically in 1989 in the Sandwich Laboratory of Pfizer Ltd, U.K. The actual aim of the drug discovery program was to discover an antihypertensive or antianginal drug. Nitrates were first line of treatment back then in 1980s for the angina pectoris. But, unfortunately after chronic usage of nitrates, it was found to develop tolerance, which limits nitrates' utility in treating angina. Hence, the scientists were thinking of an alternative way to treat angina. Phosphodiesterase (PDE) enzyme was selected as the target. It was hypothesized that if the PDE enzymes can be inhibited, the breakdown of cyclic nucleotides can be prevented, and this would result in the smooth muscle relaxation to prevent angina in the heart. PDE5 enzyme was chosen as the potential target because this enzyme was found to break down cGMP selectively. Eventually, when the sildenafil citrate was synthesized, it was chosen for further preclinical and clinical studies. In the clinical studies, it was found to develop an adverse reaction of penile erection, which led to the discovery of the most important drug for treating Erectile Dysfunction (E.D.) [25, 26].

It was previously hypothesized by the scientists and other relevant associates that the PDE 5 would be the most potential target for the therapy of several cardiovascular diseases. On the other hand, cGMP was abundant in the cytoplasm of the cells of corpus cavernosum. The first reported PDE5 inhibitor was Zaprinast (a in Table 1) found in the literature. It was developed as an anti-allergic agent. However, it has an extremely poor selectivity and weak activity. Due to its PDE inhibitory property, it was found to be a vasodilator and in dog model it was found to reduce B.P. This compound was screened against PDE5 isolated from rabbits, but it has just moderate affinity for the PDE5 enzyme. However, this initial compound gave a starting point for the medicinal chemists aiming to increase potency and selectivity in a rational drug development program. Zaprinast and cGMP were subjected to the determination of dipole moment to indicate their electronic distribution and they both provided similar electronic distribution. This indicates that the PDE5 can bind both the compounds in an analogous manner. X ray structure of Zaprinast was determined and it was found that there is a H bond between the phenyl oxygen and the pyrimidinone hydrogen atom. This indicates co-planarity; hence, this part of the molecule was kept untouched [27].
Several novel compounds were experimented against PDE enzymes to improve the potency and selectivity. The propyl group of Zaprinast was replaced with the ethyl group. Derivatization at pyrazolo[4,3-d] pyrimidin-7-one gave better potency in PDE5 inhibition, about 10 times increase in potency. PDE1 and PDE3 were also collected from animal models and tested against the compound b [28].

Table 2: Enzyme inhibitory data (IC50) for compound a and b

IC_{50}	a (Zaprinast)	b
PDE 1	9.4 µM	3.3 µM
PDE 3	> 100 µM	> 100 µM
PDE 5	2 µM	0.330 µM

The PDE 5 specifically binds with cGMP. So, it is obvious that any compound mimics the 3-dimensional structure of cGMP would bind with PDE 5 with better affinity and selectivity. The aim of increasing potency and selectivity was experimented with substituents that mimic spatial structure like cGMP. The chemical structure of compound b suggested that the pyrazolo pyrimidinone nucleus can mimic the guanosine base of cGMP [28].
Two more compounds were produced from the compound b for the intention to mimic the structure of cGMP. First, an n-propyl group was introduced in the 3-position replacing the methyl group to synthesize compound c. The replacement increased the potency in inhibiting the PDE5 enzyme. However, removal of 1-methyl group from the compound c gave the introduction of compound d. But it shows reduced PDE5 blocking activity [28].

Table 3: Enzyme inhibitory data (IC50) for compound c and d

IC_{50}	c	d
PDE 1	0.79 µM	0.86 µM
PDE 3	> 100 µM	> 100 µM
PDE 5	0.027 µM	0.082 µM

The position of 2` substituents were examined using some functional groups including hydrogen (e), hydroxyl (f), sulphonamide (i) etc. Compound e showed about 200 times reduction in potency and compound f showed 40 times reduction in potency. Others were very less potent compared to the compound c [28].

Table 4: Enzyme inhibitory data (I.C. 50) for compound e-i

Compound	**IC_{50} for PDE5 (Platelets)**
c	0.027 µM
e	4.5 µM
f	1 µM
g	0.96 µM
h	4.4 µM
i	0.78 µM

The solubility profile of the compound c was determined, and it was found to have poor aqueous solubility (log D=4.00). To make it an oral drug, the solubility should be optimized. Aiming the solubility, several

analogous compounds were made by the modification at the 5' position of the phenyl ring. The scientists tried here to mimic the ribose sugar of cGMP. Several sulphonamide derivatives (j, k, l, and m) were evaluated for their solubility and biological activity. All of them showed better aqueous solubility compared to compound c and finally compound j (sildenafil) was selected for the further preclinical and clinical studies due to its satisfactory balance in enzyme inhibition, selectivity profile, potency and solubility [28].

Table 5: Enzyme inhibitory data (I.C. 50) and solubility profile for compound c, j-m

Compound	**IC_{50} for PDE5 (Platelets)**	**LogD**
c	0.027 µM	4.0
j (Sildenafil)	0.0036 µM 0.003 µM (corpus cavernosum)	2.7
k	0.0019 µM	2.0
l	0.0021 µM	2.3
m	0.0057 µM	1.5

4. Animal P.K. Data:

The drug of our interest is slightly basic (pKa=6.5) in nature, and it is expected to highly ionize in the stomach's acidic condition and slightly ionize in the alkaline situation of intestines. Moreover, the log D value of 2.7 indicates that, it is moderately water-soluble. These optimum physicochemical properties help sildenafil to be absorbed completely from the GIT [29].

Single intravenous bolus doses were administered to different animals including men to determine the IV PK data of sildenafil. The sildenafil showed 91ml/min/kg plasma clearance for the mouse model. In the male and female rat model, the clearance was different (48 and 13ml/min/kg respectively). In the dog model, the total plasma clearance value was 12ml/min/kg. The volume of distribution in male and female rat and mouse were 1.1, 2.0, 1.0, respectively. However, the Vd was about 5.21/kg in the dog model, indicating higher tissue distribution than other tested animal models. Another implication of higher Vd in dogs is the longer elimination half-life in dogs (5.2 hours). Because, from the fundamental concept of pharmacokinetics, we know that the elimination half-life is directly proportional to the volume of distribution. On the other hand, those animal model in which clearance is higher exhibited lower half life and greater elimination rate (mouse, male rat elimination half-life less than 1 hour) [29].

Table 6: P.K. data of sildenafil in mouse, rat and dog after IV bolus administration [29]

Parameter	**Mouse (n=5)**	**Male Rat (n=3)**	**Female Rat (n=3)**	**Dog (n=5)**
IV dose (mg/kg)	1	4	4	1
Elimination half life	Not done	0.30.1	1.90.1	5.22.1
Total plasma clearance (ml/min/kg)	91	4811	131	124
Volume of Distribution (L/kg)	1	1.10.4	20.2	5.21.6

In dog model, the urinary excretion of sildenafil was about 2.8%. So, it can be concluded that most drugs are cleared via non renal mechanism. After oral dosing in different animal model certain parameters were determined including Cmax, tmax, AUC and oral bioavailability. The tmax value was minimum in female rats (0.25 hour) and maximum in mice (3 hours). They exhibited different bioavailability and AUC values for the same oral dose of 1 mg/kg in both male and female rate. This suggests the nonlinearity or capacity limited metabolism or elimination in female rats. The elimination half-lives after oral dosing were determined and observed values were slightly higher than those observed in IV bolus dosing. This phenomenon indicates that the oral absorption rate determines the overall elimination kinetics of this drug in these animal models [29].

Table 7: P.K. data of sildenafil in mice, rat and dog after oral drug administration [29]

Parameter	Mouse (n=5)	Male Rat (n=3)	Female Rat (n=3)	Dog (n=5)
Oral Dose (mg/kg)	10	1	1	1
Cmax (ng/ml)	30	16	136	11766
tmax (hour)	0.5	3.0	0.25	1.10.5
Elimination half-life (hour)	1.3	0.4	0.9	6.15.8
Oral Bioavailability (%)	17	23	44	5413

Dose dependent P.K. profile was evaluated using different animal model to determine the linearity of P.K. parameters with dose. In male rat, upon increasing 45-time dose, the increase in Cmax was 30 times and the increase in AUC was 43 times. In dog model, the increase in Cmax and AUC were 13 times and 22 times respectively upon 20 times dose elevation. However, when in female rats 45 dose was increased, the AUC was found to be greater than 450 times, and it also indicates capacity limited elimination at higher dose in female rats [29].

Table 8: Dose dependent P.K. profile

Species	Oral Dose (mg/kg)	Cmax (ng/ml)	AUC (ng.hr/ml)
Male rat (n=2)	1	16	54
Male rat (n=3)	45	477	2300
Female rat (n=2)	1	136	252
Female rat (n=3)	45	6620	116000
Dog (n=5)	1	117	842
Dog (n=2)	20	1570	18250

The plasma protein binding was determined in animal models. The protein binding was independent of concentration within the 0.01-10 microgram/ml. However, the mean binding in mouse, rat, rabbit, and dog were 94, 95, 91 and 86, respectively. The in vitro metabolism was determined by the rate of disappearance of sildenafil in hepatic microsomal cells extracted from different animals. The disappearance half-lives were 2, 129, 113, 38 in male rat, female rat, rabbit, and dog, respectively. In addition, the disappearance of sildenafil was evaluated to be synchronized with the appearance of a metabolite UK-103320 [29].

Sildenafil is cleared of the body by metabolism rather than excretion due to its lipophilic nature inherently. The metabolism of sildenafil depends on NADPH, and it is highly suggested that the P450 enzyme primarily metabolizes it. Because the inhibitors of p450 enzymes have been shown to increase its

elimination half-life. The major metabolizing enzymes are founded to be CYP2C9 and CYP3A4. The major metabolizing pathways are N demethylation, ring opening and hydroxylation. Several metabolites and unmetabolized sildenafil were identified in the fecal wastes of different animal models. For instance, in a HPLC experimentation, unmetabolized sildenafil was obtained in mouse, rat, rabbit and dog at 3%, 3%, 21% and 16% respectively. In HPLC chromatogram, the retention time of sildenafil was 55 minutes. A major metabolite named as UK-150564 (M9) was obtained in all the animals including rat (16%), mouse (19%), rabbit (5%) and dog (16%). This metabolite was the N, N-desethyl metabolite of sildenafil. Its retention time was approximately 41 minutes. Another metabolite labelled as M10 (UK-103320) with a retention time 48 minutes in fecal wastes of all the animal models. There were some other metabolites identified but in a very small percentage. However, UK-95340 was identified in dog feces but not in other animal models [29].

5. Animal Safety Data:

Sildenafil targets PDE5 enzyme selectively. But there is another target of sildenafil which is PDE6. But the potency is much lower for the targeting of PDE6 in animal models present in retina smooth muscles including rods and cones. But the effect of sildenafil to these targets were insignificant to that of the desired pharmacological effect [30].

Sildenafil citrate was evaluated in animal models using single dose as well as multiple dose strategies. In mice, the highest nontoxic dose was found to be 500 mg/kg; in rats, it was found to be 300 mg/kg. In repeated dose study using rats, a dose dependent increase of liver weight was observed. This was accounted for the hepatic centrilobular hypertrophy. This was not found in any other animal models. In addition, rats are sensitive to thyroid hormone metabolism because they do not have thyroxine binding globulin. This causes shorter half-life of thyroxine in rats. But this effect not relevant in humans [30].

In an extended dose model for 29 days, it was found that thyroid hormonal change can happen including the increased clearance of thyroid hormone and increased production of thyroid stimulating hormone (TSH). The maximum safe dose for rats was 60 mg/kg. In mice, there was no toxicity withing the concentration range of 10-20 mg/kg [30]

In dog animal model, the heart rate was found to be increase from the dose 10 mg/kg to 80 mg/kg. In addition, the blood pressure in both systolic and diastolic phases was reduced. However, the electrocardiogram did not display any rhythmic abnormalities in the model animals. In some of the dogs, some mile side effects were observed including emesis, transient salivation, and pupil dilation of the eyes. In the 12 months long animal model studies, most dogs suffered from one or more side effects including stiffness, stiff neck, neutrophilia, mild anemia, hypoalbuminemia, hyper-alkaline phosphatase, and lack of other electrolytes. These syndromes are cumulatively known as Beagle Pain Syndrome (BPS) which was observed at a higher dose of 50 mg/kg or higher. However, this syndrome was not observed in lower dose below 20 mg/kg indicating its safety to use in humans [30].

The sildenafil citrate is the most widely used drug prescribed for penile erection. Whether it produces any side effect affecting reproduction in male is obvious. In animal models, there was no record of impaired fertility in both male and female animals. Moreover, there was no sign of prenatal and postnatal significant abnormalities. In male and female rat models, after repeated dose of 60 mg/kg, the gonads were evaluated in both male and female rats, however there was no symbol of impaired gonad morphology and structure in cellular level. During gestation period in rats and rabbits, the effect of sildenafil was evaluated and there was no observable maternal toxicity during gestation. Therefore, it can easily be concluded that sildenafil is surely not to be a teratogenic substance [30].

Pregnant female rats were treated with sildenafil to find any post-natal toxicity associated with sildenafil. There was a minor toxic event reported at a higher dose of 60 mg/kg or higher, however this event is not significant for the human usage of sildenafil citrate. Using bacterial and mammalian cells in vitro, genotoxicity test was done, and sildenafil was found to be non-genotoxic. In carcinogenic study using mice and rats, it was observed that sildenafil is not a carcinogenic for those animals [30].

6. Animal Efficacy Data:

When sildenafil was administered intravenously in rabbit model, the intracavernosal pressure (ICP) was determined. There was no effect on ICP at lower dose up to 10 µg/ml. At a higher dose beyond that point, the ICP increased dose-dependent. Similar type of observations was found in other animal models including cats. When the drug was administered directly into the corpus cavernosum of the penis of the

rabbits, the ICP was increased depending on the dose. The drug also enhanced the duration of increased ICP and systemic B.P. The erection of the penis was caused at about 5 minutes and it lasted for about 30 minutes [31]

7. Human P.K. Data:

Sildenafil citrate is the first marketed drug for the treatment of erectile dysfunction. Therefore, significant considerations were invested for evaluating its pharmacokinetics properties and its correlation with pharmacodynamic and toxicological data. It has been mentioned earlier that sildenafil is the first selective inhibitor of phosphodiesterase type V enzyme. It has an IC50 value of around 3.9 nM. Its selective affinity for this enzyme is about 1000 times better than other member of this family (PDE2, PDE3, PDE4). However, it has moderate selectivity for PDE1 and has recently been reported for PDE6. This PDE6 affinity of the sildenafil accounts for its visual abnormalities at higher dose. It has been reported that dose higher than 200 mg daily or AUC higher than 2600 ng.hr/ml or Cmax greater than 500 ng/ml intensify the risk of visual, gastrointestinal or vascular side effects [32, 33].

Although sildenafil citrate is available in different doses containing equivalent amount of sildenafil of 25, 50 or 100 mg, it is suggested by the physicians that 50 mg should be the starting dose of any patient considering for penile erection. And the drug should be taken approximately 60 minutes before the intercourse. However, since the hepatic enzymes metabolize sildenafil, it is highly recommended to take lower dose of 25 mg for liver patients. Not only that, but older patients are also frequently advised to take lower dose. And if the patient is taking any kind of medication that inhibits or alters the metabolism of sildenafil by P450 enzymes, the dose of the drug must be matched that way [32, 33].

From the pharmacokinetic modeling principles, most drug response is highly dependent on its plasma drug concentration, especially AUC or Cmax. However, for sildenafil, there is no obvious correlation between the plasma drug concentration and the biological activity of erection including the onset of erection and the duration of erectile penis. Therefore, dose has been the best predictor of the biological activities for this drug. The average ED50 value calculated from an Emax model experimentation was around 40 mg. However, for the diabetic patients the ED50 value was found to be more than 180 mg [32, 33].

7.1 Absorption:

Being a BCS class I drug (highly soluble, highly permeable), sildenafil is rapidly absorbed from the GIT. It is lipophilic drug with pKa of 6.5, which indicates that it is partially ionized in the intestinal pH. It reaches its maximum plasma drug concentration in less than one hour with a first order absorption rate constant of 2.6/hr. The oral bioavailability in human is around 40%. However, radioactivity studies showed that about 92% drug is absorbed from the GIT. So, the lower bioavailability of this drug can be accounted for the gut wall metabolism and first-pass hepatic metabolism [32, 33].

The impact of fat type meal has been determined on the absorption of sildenafil. The tmax was 1 hour greater in case of fed model. The Cmax was also found to be reduced 29%. The total AUC was reduced 11% after the meal. These findings indicate that the food interferes with the absorption of drug from the GIT [32, 33].

To determine dose linearity, doses within the range of 25-200 mg were administered and the gradual increase in systemic exposure and Cmax were calculated. In this range, the increase in AUC was quite linear. For instance, the AUC values for 25, 50, 100 and 200 mg doses were 361, 738 (2-fold), 1685 (2.3-fold) and 3755 (2.3-fold) ng.hr/ml. However, the linearity was disrupted after the 200 mg dose range. For example, when the dose was increased from 100 mg to 800 mg by 8-fold increase, the AUC was found to be increased by 15-fold. This finding suggests that dose greater that 200 mg saturates the elimination pathway for the sildenafil and correspondingly increase the AUC and other parameters including elimination half-life. There has been no significant Pk differences in healthy volunteers and E.D. patients [32, 33].

7.2 Distribution:

Sildenafil and its major active metabolite are highly bound to plasma protein. The steady state volume of distribution of 105 L suggests that this drug is highly bound to tissue proteins and other proteins outside the plasma. In a research with the patients of E.D., the volume of distribution was found to be 310 L. A slight amount (0.001%) drug was found in semen, which is unlikely to have any biological effect in the partners of the patients [32, 33].

7.3 Metabolism:

Sildenafil citrate, when taken orally, most of the drugs are eliminated via metabolism and in the feces. Some of the drugs are also excreted through the urine. After IV administration the systemic clearance was found to be 41 L/hr. The predicted extraction ratio by the liver is 50%. Plasma concentration of both the parent drug and the metabolite decreases exponentially indicating the drug to be best fitted in two compartment P.K. model. The terminal phase half-life was found within 3-5 hours [32, 33].

The hepatic P450 enzymes highly metabolize sildenafil. CYP3A4 is the main enzyme for its metabolism. There are about 16 different metabolites identified through several pathways including N-demethylation, oxidation, and hydroxylation. The most important active metabolite is UK-103320. It accounts for about 20% of the activity of the parent drug to the PDE5. This metabolite has a tmax of 1.4 hours in fasted condition and tmax also delays in fed condition. This metabolite is seen in both cases of IV administration and oral administration, however in oral the prevalence is greater indicating the impact of first pass metabolism [32].

Another metabolite is easily identified due to 2 carbon fragment loss from the piperazine ring of sildenafil (UK 150564). However, it accounts for insignificant pharmacologic activity at PDE5 inhibition [32].

Table 9: Summary of Human P.K. & P.D. of sildenafil [32, 33]

Parameters	Value
Available doses (mg)	25, 50, 100
IC50 for PDE5 (nM)	3.5-8.5
ED50 (mg)	36-41
Oral absorption Fa (%)	92
Oral bioavailability F (%)	38-41
Tmax (hrs.)	1 (0.5-2.0)
Onset time (min)	60
Duration of action (hrs.)	4-8
Cmax (µg/L) (fasting)	560 (100 mg dose)
Food effect	tmax 1 h ↑, Cmax 29% ↓, AUC 11% ↓
Alcohol interaction	None
AUC (µg.hr/L)	1685 (100 mg dose)
Dose linearity	Moderately nonlinear
Vss (L)	105
Protein binding (%)	96
% Dose found in semen	<0.001
CYP isoenzymes	CYP3A4 (79%), 2C9 (20%), 2C19, and 2D6 < 1%
Active metabolites	N-desmethyl sildenafil (50% potency; 20% contribution to activity)
Excretion	Feces major (73–88%), urine minor (6–15%)
Renal excretion of unchanged drug (%)	<1
CL (L/hrs)	41
Variability in CL/ F	29% between-subject
T ½ (hrs)	3-5

7.4 Drug Interactions:

CYP3A4 is the principle-metabolizing enzyme for the sildenafil which account about 79% metabolism of the drug. Other enzymes include CYP2C9 and CYP2C29. However, inhibitor of later two enzymes did not significantly inhibit sildenafil metabolism, but the CYP3A4 inhibitors including ketoconazole, ritonavir, and erythromycin severely decrease the metabolism of sildenafil and increase their systemic exposure [32, 33].

In an experiment, erythromycin was coadministered with sildenafil. The AUC and Cmax were elevated more than twice however the elimination half-life were somehow unchanged. This indicates that the systemic availability of erythromycin is inhibiting CYP3A4 to metabolize the sildenafil and reducing its first

pass and gut wall metabolism, but it has no impact on the systemic clearance of the drug. From the different findings it seems that, at higher concentration of sildenafil in plasma, it is primarily metabolized by high capacity, low affinity enzyme CYP3A4. That is why erythromycin impacts the first pass metabolism due to higher concentration of sildenafil. However, when the concentration is low, the drug is metabolized by low capacity, high affinity enzyme CYP2C9. Since, this enzyme is not inhibited by erythromycin, it has no impact on elimination half-life of the drug [32, 33].

Ritonavir is an antiretroviral protease inhibitor which inhibits the action of CYP3A4. 500 mg twice a day dose has been found to alter the pharmacokinetics of the sildenafil. AUC increased 11-fold, Cmax increased 3.9-fold and tmax was delayed for 3.1 hours. Saquinavir is another antiretroviral protease inhibitor which also inhibits the action of CYP3A4. 1200 mg thrice a day dose has been found to alter the pharmacokinetics of the sildenafil. AUC was increased 3.1-fold, Cmax increased 2.4-fold, and tmax was delayed for 2.6 hours. In both cases, there was no impact on the elimination 7half-life for the same reason discussed in the erythromycin case [32, 33].

Grapefruit juice is known to inhibit the enzymatic action of CY3A4. It has been found to alter the pharmacokinetic data of sildenafil. Simultaneous ingestion of this juice with the drug has increased the systemic exposure and delayed the tmax. However, the Cmax was not changed [32, 33]

The cimetidine has investigated nonspecific inhibition of CYP enzymes. In this case, tmax and elimination half-life was also unchanged, but the AUC and Cmax was changed significantly. This suggests that the nonspecific CYP inhibitor blocked the first pass metabolism in the liver and the gut [32, 33].

Other metabolizing enzymes, if inhibited, it can increase the systemic exposure of sildenafil. Fluvoxamine, the inhibitor of CYP2C9, when coadministered, increased the AUC by 37% [32, 33].

7.5 P.K. Data on Special Populations:

Effect of Age:

In elderly healthy volunteers' sildenafil has been cleared more slowly than the young volunteers. This has been attributed to decreased activity of the hepatic enzymes and increased plasma protein binding in elder people. Another investigation suggested that every decade age increase reduces 6% systemic clearance of the drug [32, 33].

Effect of Race:

Mexican males were found to have greater oral bioavailability and systemic exposure due to reduced enzymatic activity of CYP3A4, however half-life was same.

Effect of Uremic Condition:

Renal impairment and insufficient renal activity are linked with reduced clearance of sildenafil and increase in the exposure of the drug systemically. But, sildenafil or its metabolites are excreted in the urine in a very low concentration, so the effect should not be direct, rather indirect effect of the renal condition on the hepatic metabolism of the drug should be the exact reason [32, 33].

Effect of Hepatic Condition:

In hepatic disease line child pugh A and B, sildenafil clearance was reduced significantly which caused a great increase (84%) in AUC of the parent drug. Cmax was increased by 47% and t1/2 was delayed by 34%. It was observed that the impact of hepatic failure is more obvious on the metabolite clearance than that of the parent drug [32, 33]

4. CONCLUSION

After the completion of the lengthy process of drug discovery, sildenafil citrate was approved by the FDA to be used for erectile dysfunction in March 1998. It was the very first oral treatment for the erectile dysfunction. Although several selective PDE 5 inhibitors are available now in market, however sildenafil citrate is still one of the most prescribed drugs for the treatment of penile erectile disorders.

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