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ORAL PHARMACOKINETICS OF SILDENAFIL IN THE PRESENCE OF CYP3A4-INDUCER AND -INHIBITOR USING CANNULATED AND NON-CANNULATED MALE SWISS ALBINO MICE

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ABSTRACT

OBJECTIVE: Sildenafil is majorly metabolized by Cytochrome P450 (CYP450) isoform 3A4. The latter is responsible for the metabolism of more than 50% of the drugs. It is involved in multiple clinically relevant drug-drug interactions. To evaluate the mice pharmacokinetics model as a drug- drug interaction model by using Sildenafil in the presence of Carbamazepine (CYP3A4 inducer) and Ketoconazole (CYP3A4 inhibitor) in male Swiss albino mice. Also, efforts have been put-forth to employ cannulated mice and compare the data with the non-cannulated mice.

METHODOLOGY: Ketoconazole and Carbamazepine (10 mg/kg, PO) were dosed for 6 days in different groups of mice. On day 6 Sildenafil (10 mg/kg, PO) was dosed 15 minutes post Ketoconazole and Carbamazepine administration. Two control groups were dosed with Sildenafil alone. Blood samples were collected through retro-orbital plexus for sparse sampling and jugular vein for cannulated mice at 0.25, 0.5, 1, 2, 4 and 8 h post Sildenafil administration and analyzed by LC-MS/MS.

RESULTS: Ketoconazole increased the C_{max} and AUC_{last} of Sildenafil by 146% and 219% in non-cannulated and 63% and 194% in cannulated mice respectively, as compared to control group.

Carbamazepine decreased the C_{max} and AUC_{last} of Sildenafil by 36% in non-cannulated mice and 61% and 58% in cannulated mice respectively, as compared to control group. There was no significant difference in the pharmacokinetic parameters of Sildenafil in cannulated and non-cannulated groups.

The study reflects to the underlying drug-drug interactions which can attribute in the pharmacokinetics of Sildenafil, in the presence of drugs, which are inducers and inhibitors of CYP3A4. Also, that cannulated mice can be employed to reduce the animal usage by one third without compromising the data quality.

KEYWORDS : Sildenafil, CYP3A4 inhibitor, CYP3A4 inducer, Ketoconazole, Carbamazepine.

INTRODUCTION

Sildenafil (Viagra™, 1- [4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1Hpyrazolo [4,3-d] pyrimidin-5-yl) phenylsulphonyl]-4-methyl piperazine) was introduced by Pfizer Inc. in 1998 for the treatment of erectile dysfunction (ED) [1]. Sildenafil has no direct relaxant effect on isolated human *corpus cavernosum*; but increases the effect of nitric oxide (NO) by inhibiting phosphodiesterase type V (PDE V) has vital role in the degradation of cGMP in the *corpus cavernosum* which results in smooth muscle relaxation of penis [2]. Erectile dysfunction (ED) is very common in men suffering with renal disease and with increased age [3]. The change in clearance of Sildenafil alter its pharmacological impact significantly [3]. Numerous medications which are taken ordinarily may modify the elimination/clearance and AUC of Sildenafil by hindering and prompting the CYP enzymes [4]. More than 30% deaths are reported in men with erectile dysfunction prescribed with Sildenafil [5]. Side effects of Sildenafil like cardiovascular, ocular can be enhanced by increase in AUC [5, 6]. It is accounted for that Sildenafil is significantly metabolised by CYP3A4 and to a lesser degree by CYP2C9 [7, 8] and the enzyme inhibition phenomena is more frequent than enzyme induction [9].

Cytochrome P450 (CYP) system comprises of a superfamily of haemoproteins that metabolises large number of xenobiotic and endobiotic [9, 10]. It plays an important role in phase-I metabolism of many drugs. The maximum drugs that experience CYP mediated biotransformation are responsible for the huge number of clinically significant drug interactions during polypharmacy. Drug-drug interactions showcase a major concern from drug discovery to post market surveillance. Although Cytochrome P450 consists of more than 200 isoenzymes, six of them metabolize 90 percent of drugs, with the two most prevalent enzymes being CYP3A4 and CYP2D6. CYP3A4 is an important enzyme in the body, available inliver and intestinal epithelium, responsible for metabolism of more than 50% drugs. CYP3A4 is involved in many clinically important drug-drug interactions. Unfortunately, numerous CYP3A4 substrates have significant toxicity and few patients may develop severe toxicity when CYP3A4 inhibitors are taken simultaneously [11, 12].

Variety in CYP isoforms among various species is very much archived, be that as it may, numerous reports exist exhibiting similarities of rodents CYP3A with that of human CYP3A4 [13].

Pharmacokinetics with sparse sampling having few drawbacks, can be excluded by serial sampling (whole blood, 25- 40µL). Serial sampling gives more reliable data [14] and decrease the animal numbers [15],

animal stress and cost of the study. Carbamazepine is an inducer of several CYPs- 1A2, 2C9, and 3A4, as well as the active transporter P-glycoprotein. Hence the drugs metabolised via CYP1A2, CYP2C9, CYP3A4 or a substrate for the P-glycoprotein transporter, can be affected by Carbamazepine. Further, several clinical and pre-clinical studies revealed that Ketoconazole is strong inhibitor for CYP3A4 substrates [8- 11].

In this study Carbamazepine was taken as inducer and Ketoconazole as inhibitor [16] to understand their effect on the pharmacokinetics of Sildenafil in cannulated and non-cannulated Swiss Albino mice.

MATERIALS AND METHODS

MATERIALS:

Ketoconazole (Cat. No. K1003), Carbamazepine (Cat. No. C8981), K_2EDTA (Di-potassium ethylenediaminetetraacetic acid) (Cat No. 03660) and Telmisartan (Cat. No. T 8949) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Sildenafil Citrate (CAS: 171599-83-0) was procured from Labex Corporation, New Delhi. HPLC grade acetonitrile, methanol, water and formic acid (90% pure) were purchased from Merck specialities Pvt Ltd (Mumbai, India). Silicon tubing for cannulation was procured from Bio-Sci Instruments, Mumbai. Mouse harness were procured from Instech Laboratories, Inc. USA.

ANIMALS:

The study protocol was approved by IAEC (Institutional Animal Ethics Committee) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Male Swiss Albino mice were procured from Hylasco Bio-Technology Pvt Ltd. Hyderabad (Distributors for Charles River Animals) and were subjected to three days quarantine period in AAALAC and OLAW accredited vivarium. Animals were maintained and monitored for good health in accordance with Test Facility SOPs and at the discretion of the Laboratory Animal Veterinarian (LAV). Normal rodent pellet feeds (Safe Diet procured from Samitek Instruments) was provided. Water was available *ad libitum*. Environmental controls for the animal room were set to maintain a temperature of 22±2℃, humidity of 40-70% relative humidity, and a 12-hour light/12-hour dark cycle. Normal healthy animals certified by the Laboratory Animal Veterinarian (LAV) were selected and acclimatized for minimum three days prior to initiation of study. Animals were divided into six groups.

MICE JUGULAR VEIN CANNULATION:

Mice were anaesthetized using isoflurane, and anaesthesia was maintained using isoflurane dispensing assembly (EZ Anesthesia Machine). A cannula of silicone tubing with an internal diameter (ID) of 0.3 mm and an outer diameter (OD) of 0.6 mm was used to cannulate the animals for group 2, 4 and 6. Right Jugular vein was exposed, and a loose ligature was placed caudally, and cranial end of vein was ligated. A small incision was made between the ligatures into which the catheter was inserted. The catheter was secured in place by tying the loose ligature around the catheterized vessel. A small incision was made in the scapular region to serve as the exit site of the catheter. The catheter was subcutaneously tunneled and exteriorized through scapular incision. A stay suture was tied in the scapular area. The incision was sutured with sterile suturing material. Anti-septic solution was applied to the sutured site Mouse harness was harmonized in forearm of mice and scapular region [17]. Patency was tested, and catheter was filled with a locking solution (heparinized saline) and sealed with a stainless-steel plug. Animals were closely observed throughout the recovery period.

PHARMACOKINETICS:

Mice of Groups 1 (non-cannulated) and 2 (cannulated) were dosed with water for injection (vehicle control), groups 3 (non-cannulated) and 4 (cannulated) were dosed with Ketoconazole (inhibitor) and groups 5 (non-cannulated) and 6 (cannulated) were dosed with Carbamazepine (inducer). On sixth day all the groups were dosed with vehicle, inhibitor and inducer 15 min before Sildenafil. Animals were dosed at 10 mg/kg with dose volume of 5 mL/kg. Whole blood was used for Sildenafil analysis. Immediately after collection samples were stored at 4ºC. Sildenafil formulation was prepared in water for injection and other two (Ketoconazole and Carbamazepine) formulations were prepared with Tween 80 (1% v/v) + 0.5% Methylcellulose (99% v/v). Please refer to the Study design (Figure 1).

BIOANALYTICAL METHOD

Preparation of calibration and quality control samples

Master stock solutions of Sildenafil Citrate, Telmisartan (Internal Standard: IS) were prepared in DMSO at 1 mg/mL concentration. Working standard solutions of Sildenafil were prepared by serial dilution from master stock in DMSO: methanol: water (20:40:40, v/v) proportions respectively. Working standard solutions were prepared at 25-fold higher concentrations to achieve the final extracted blood calibration and quality control samples concentrations. A total of nine calibration standards and three quality control samples were prepared. Calibration standards (1.03, 2.05, 10.26, 51.32, 205.26, 513.16, 821.05, 922.53, 1025.03 ng/mL) and quality control samples (4.1, 512.52, 854.19 ng/mL) of Sildenafil were prepared in blood by spiking 1 µL of the working standard solution in to 24µL of blank blood. The working solution of IS (200 ng/mL) was prepared by dilution of an aliquot of master stock solution with acetonitrile. All Sildenafil Citrate and Telmisartan solutions were stored at 4°C in polypropylene tubes.

SAMPLE PREPARATION

A 25 µL aliquot of blood (samples from Pharmacokinetic study) were transferred in to a 96 well polypropylene plate and Sildenafil was extracted with acetonitrile containing internal standard. Samples were vortex mixed for 5 minutes at 1000 rpm and centrifuged at 2500g for 5 minutes at 4°C. Supernatant (100 µL) was separated and transferred in to a fresh plate for analysis and diluted with 150 µL of methanol: water (1:1, v/v), from this 10 µL was injected for LC-MS/MS (Liquid chromatography-tandem mass spectrometry) analysis.

LC-MS/MS ANALYSIS

All mass spectrometric detections were performed on API-4500 triple quadrupole instrument (SCIEX)) with a Turbo V™ ionisation source interface. Data acquisition and processing for quantification were performed using Analyst software version 1.6.3 (SCIEX). The mass spectrometric conditions were optimized for the compounds by infusing a 500 ng/mL solution in methanol: water (1:1, v/v) at 10 µL/minute flow rate using Harvard infusion pump (Harvard Apparatus, Holliston, USA) connected directly to the mass spectrometry. Flow dependent source parameters were optimised by flow injection analysis (FIA) with 1.00 mL/minute flow rate of mobile phase without column. The Turbo V source with ESI (Electro spray ionization) probe was operated with optimised settings as follows: polarity: positive, curtain gas: 20 psi, nebuliser gas (GS1): 50 psi, heater gas (GS2):55 psi, ion spray voltage: 5500V, source temperature: 550° C. The mass spectrometry was operated in MRM mode in which both parent ion and fragment ion are fixed. The m/z value of Sildenafil parent and fragment ions used were 475.2 and 283.10 with optimum declustering potential (DP) and collision energy (CE) of 120 V and 48 V respectively. For Telmisartan 515.1 and 276.100 were the m/z values used for parent and fragment ions with DP and CE of 60 V and 60 V respectively.

The LC system used was Sciex Exion consisting of two isocratic pumps, a vacuum degasser, and a temperature-controlled AD Multiplate Autosampler set at 4°C and a thermostatic column oven set at 40° C. The stationary phase used for the chromatography was Kinetex EVO, C18 with 5 µm particle size and dimensions of 50×4.6 mm (Phenomenex, US). The mobile phase consisting of 0.1% formic acid in water (aqueous reservoir) and 100% methanol (organic modifier) was used at flow rate of 1.0 mL/minute. A common reverse phase gradient programme [time (min) / % B= 0.01/5, 1.0/95, 2.40/95, 2.50/5, 3.50/5] was used with a short run time of 3.5 minutes (Figure 8).

PHARMACOKINETIC PARAMETER ANALYSIS:

Pharmacokinetic parameters were calculated for the mean blood concentration of group 1, 3 and 5 (non-cannulated) and individual mice blood concentration for group 2, 4 and 6 (cannulated) by Non-compartmental model, Linear Log Trapezoidal (200-202), with Phoenix software version 7.0.

STATISTICAL ANALYSIS

Below P value 0.05 was considered to be statistically significant using an unpaired t-test. The statistics was applied in cannulated groups of mice, as in the non-cannulated groups PK parameters were calculated with mean blood concentration (composite sampling).

RESULTS

A substantial increase of 219% (2289 ng·h/mL) and decrease of 36% (593 ng·h/mL) in $AUC_{0\text{-last}}$ for group 3 and 5 respectively, was observed when compared with group 1 (947 ng·h/mL). The PK parameters were analysed with mean values (sparse sampling) therefore statistical analysis was not performed for non-cannulated groups. (Table 1,

Figure 2). Further, in cannulated groups there was significant increase of 194% (3264 ng·h/mL) and decrease of 59% (469 ng·h/mL) in AUC_{0-last} respectively, for group 4 and 6 (Figure 7) when compared with group 2 (1112 ng·h/mL). (Table 2, Figure 3).

C_{max} (ng/mL) of Sildenafil for non-cannulated groups 1, 3 and 5 was 930, 2289 and 593 ng/mL respectively. Similarly, with cannulated groups 2, 4 and 6, C_{max} of Sildenafil was 1262, 2052 and 494 ng/mL respectively. There was measurable difference in C_{max} of Sildenafil, with inhibitor and inducer in both cannulated and non-cannulated groups

Significant difference was not observed for C_{max} and AUC_{0-last} between cannulated and non-cannulated respective groups (Table 1 and 2, Figure 4- 6).

Table 1: Pharmacokinetic parameters of Sildenafil in the presence of Ketoconazole and Carbamazepine after oral administration in non-cannulated mice at 10 mg/kg

PK Parameters	Non-cannulated		
	Sildenafil	Sildenafil + Ketoconazole	Sildenafil + Carbamazepine
Groups	1	3	5
Dose (mg/kg)	10.00	10.00	10.00
C_{max}(ng/mL)	929.87	2288.77	592.82
T_{max} (h)	0.25	0.50	0.25
AUC_{0-last} (ng·h/mL)	947.19	3024.50	607.03
AUC_{0-inf} (ng·h/mL)	948.57	3029.42	659.68
AUC_{Extra} (%)	0.15	0.16	7.98
MRT_{0-last} (h)	1.14	1.22	1.71

Animals for groups 1, 3 and 5 were dosed with vehicle (water for injection), Ketoconazole (10 mg/kg), and Carbamazepine (10 mg/kg) for 6 days respectively. On day 6 Sildenafil (10 mg/kg) was dosed in all the groups after 15 minutes. C_{max}: maximum concentration, T_{max}: time at which maximum concentration was observed, AUC_{0-last}: area under the curve for all blood time points. AUC_{0-inf}: area under the curve from time zero extrapolated to infinite time, MRT: mean residence time.

Table 2 Pharmacokinetic parameters of Sildenafil in the presence of Ketoconazole and Carbamazepine after oral administration in cannulated mice at 10 mg/kg

PK Parameters	Cannulated		
	Sildenafil	Sildenafil + Ketoconazole	Sildenafil + Carbamazepine
Groups	2	4	6
Dose (mg/kg)	10.00	10.00	10.00
C_{max}(ng/mL)	1261.66±585.45	2052.27±241.93	493.98±153.66
T_{max} (h)	0.5#	0.25#	0.25#
AUC_{0-last} (ng·h/mL)	1111.59±191.78	3263.53±449.07*	469.06±112.02*
AUC_{0-inf} (ng·h/mL)	1150.17±201.50	3313.65±452.39	502.57±107.22
AUC_{Extra} (%)	3.27±3.03	1.50±1.74	6.78±7.55
MRT_{0-last} (h)	0.98±0.28	1.71±0.28	1.69±0.34

Note: #T_{max} mentioned as median. All, the vales are mentioned as Mean±SD except T_{max}. Animals for groups 2, 4 and 6 were dosed with vehicle (water for injection), Ketoconazole (10 mg/kg), and Carbamazepine (10 mg/kg) for 6 days respectively. On day 6 Sildenafil (10 mg/kg) was dosed in all the groups after 15 minutes. C_{max}: maximum concentration, T_{max}: time at which maximum concentration was observed, AUC_{0-last}: area under the curve for all blood time points. AUC_{0-inf}: area under the curve from time zero extrapolated to infinite time, MRT: mean residence time.

*Significant difference was observed in AUC_{0-last} for group 4 (p value = 0.0016) and 6 (p value = 0.0074) when compared with group 2 using unpaired t-test.

Figure 1. Detailed Study Plan.

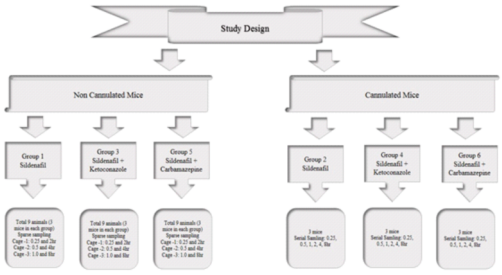


Figure 1: Schematic diagram of study design,

Figure 2. Concentration-time profiles of Sildenafil alone and in the presence of Ketoconazole and Carbamazepine after oral administration in non-cannulated mice at 10 mg/kg

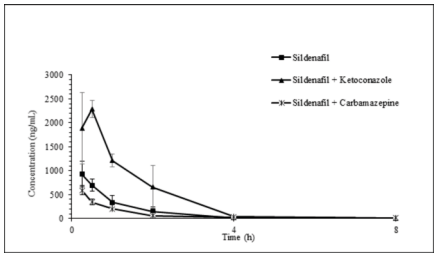


Figure 3: Concentration-time profile of Sildenafil alone and in the presence of Ketoconazole and Carbamazepine after oral administration in cannulated mice at 10 mg/kg.

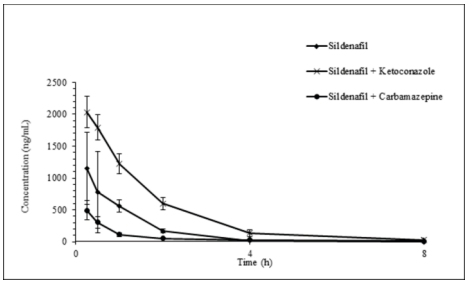


Figure 4: Concentration-time profile of Sildenafil after oral administration in cannulated and non-cannulated mice at 10 mg/kg.

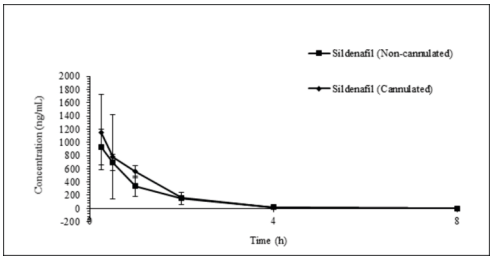


Figure 5: Concentration-time profile of Sildenafil in presence Ketoconazole after oral administration in cannulated and non-cannulated mice at 10 mg/kg

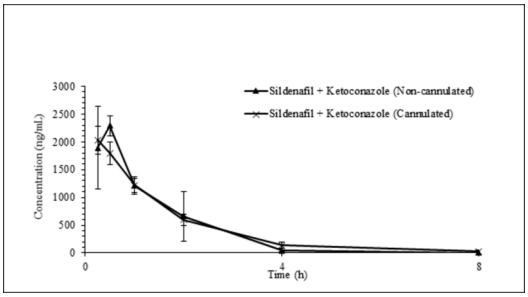


Figure 6: Concentration-time profile of Sildenafil with Carbamazepine after oral administration in cannulated and non-cannulated mice at 10 mg/kg.

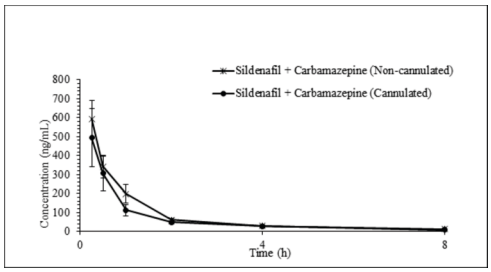
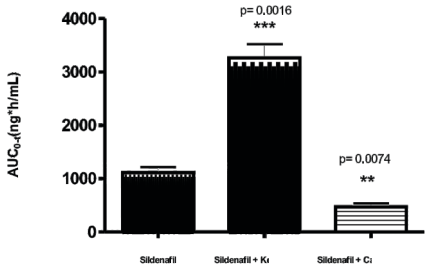
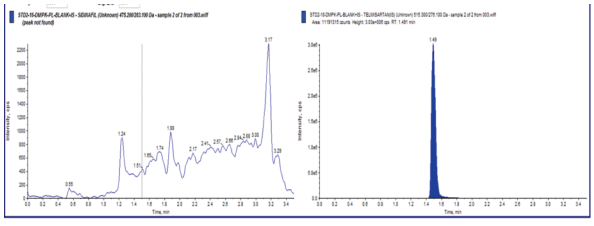


Figure 7: Statistical analysis of Sildenafil, Sildenafil + Ketoconazole and Sildenafil + Carbamazepine after oral administration in cannulated mice at 10 mg/kg.

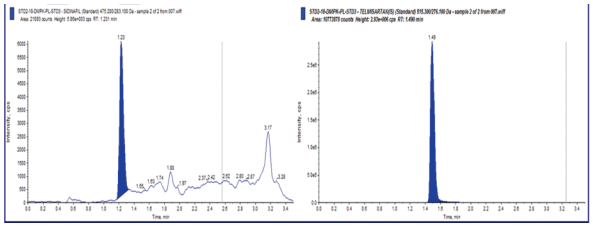


***Significant difference was observed in $AUC_{0\text{-last}}$ for group 4 (p value = 0.0016) and** for group 6 (p value = 0.0074) when compared with group 2 using unpaired t-test. $p<0.05$ was considered significant.

Figure 8: Sildenafil LC-MS/MS chromatograms
1.Extracted blank + IS (Internal Standard)



2.Extracted standard



DISCUSSION:

The age of coursing medication (and metabolite) focuses in experimental animals and assessment of PK/TK (Toxicokinetic) during drug discovery and advancement is central to set up the movement of analyte(s) against the target (or targeted ailment) as well as toxicity and safety. Furthermore these information are utilized to create in silico models, comprehend *in vitro-in vivo* extrapolations, and at last in human PK forecasts. Since concomitant administration of two or more drugs is common practice for chronic diseases and complications, we have to consider it prudent to minimize the potential for drug-drug interactions (DDI) amongst them.

There are several techniques have been published portraying the forecast of DDI based on in vitro information, true inhibitor concentrations in the liver and the digestive system can't be correctly anticipated on plasma concentration or dosages alone. Study suggests 'a fruitful extrapolation of *in vitro* information to the *in vivo* circumstance in preclinical investigations should provide a decent forecast in human studies, and *in vivo* models could be utilized as an additional tool. In the present study, the effect of Ketoconazole (CYP3A4 inhibitor) and Carbamazepine (CYP3A4 inducer) was studied on the pharmacokinetics of Sildenafil. Further, we investigated the difference in the pharmacokinetics of Sildenafil in cannulated (serial sampling) and non-cannulated (sparse sampling) mice.

Sildenafil was developed as an orally active vasodilator that extends the effect of cGMP by selectively inhibiting PDE5 in blood vessels. Its action is not restricted only to peripheral tissues. The drug crosses the blood–brain barrier and exerts versatile central effects like various neurologic disturbances, amnesia, and aggressive behaviour [18, 19] thereby, demanding to conduct DDI studies. Sildenafil is metabolized mainly by the cytochrome P450 enzyme 3A4 [20- 22], which is the principle enzyme responsible for the oxidative metabolism of the majority of current drugs. Clinical research on strong inhibitors of cytochrome P450, specifically, co-administration of Sildenafil with potent 3A4 inhibitors such as azole antifungal agents, macrolide antibiotics, and protease inhibitors, recommend carefulness with dosing [20, 23]. Even inhibitors of CYP3A4 that exist in grapefruit juice have been shown to alter Sildenafil bio-metabolism [24].

Few studies have been conducted in mice demonstrating the effect of Ketoconazole on CYP3A4, mimicking human conditions [25- 27]. However, not much work exist demonstrating the effect of CYP3A4 inhibitor and inducer on the pharmacokinetics of Sildenafil in mice. To the best of our understanding this the maiden work presenting the effect of Ketoconazole and Carbamazepine on the pharmacokinetics of Sildenafil in mice. To have the optimal enzyme inhibition and induction, mice were dosed for 6 days with CYP3A4 inhibitor (Ketoconazole) and inducer (Carbamazepine) and their effect on the pharmacokinetics of Sildenafil was evaluated. The results suggest that there is significant difference in the exposure levels of Sildenafil in presence and absence of inhibitor and inducer. This is in alignment with the various reports of *in-vitro* drug-drug interaction studies [21, 28].

Several studies have been conducted to determine the pharmacokinetics of a compound by micro sampling in mice using Dry Blood Spot (DBS) [29, 30] and whole blood [31]. Due to high variability and comprehensive method optimization processes involved in DBS card, this is likely to have high variability and is also time consuming [32]. To address these demerits of DBS, we have established and employed a serial blood sampling method in jugular vein cannulated mice and used blood sample. To revoke low sample volume (40 uL) due to restriction in the blood volume withdrawn from mice within 24 h, a high sensitive bioanalytical method was developed. The benefits of our method are (1) with cannulated mice, exact volume of blood has been taken at each time point through syringes without any blood loss, (2) anaesthetic agent is not mandatory during sampling, (3) no physical restraining of animals during blood collection. We obtained similar results in the cannulated and non-cannulated mice.

CONCLUSION:

The finding of this study represents that mice model can be used as the first level of screening to demonstrate the drug-drug interactions between two or more co-administered compounds at preclinical stage itself. This will save a lot of time and resources and facilitate a quick go or no go decision. Ketoconazole (inhibitor) and Carbamazepine (inducer) treatment resulted in decreased and increased clearance of Sildenafil in mice respectively.

There was no significant difference observed between the cannulated and non-cannulated mice. So we can use cannulated mice to minimise the number of animals per study. Further, we demonstrated that the pharmacokinetic parameters of Sildenafil corroborates for both serial and discrete sampling. It is consistent with the 3Rs principles by achieving significant reduction in the number of animals used, decreased animal stress and improved data quality.

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REFERENCES:

1. Walker DK, Ackland MJ, Jamesoe GC, Muirhead GJ, Ranceoe DJ, Wastall P, Wright PA. Pharmacokinetics and metabolism of sildenafil in mouse, rat, rabbit, dog and man. Xenobiotica. 1999; 29: 297 - 310.
2. Umrani DN, Goyal RK. Pharmacology of Sildenafil Citrate. Indian Journal of Physiology and Pharmacology. 1999; 43: 160-164.
3. Grossman EB, Swan SK, Muirhead GJ, Gaffney M, Chung M, Deriesthal H, Chow D, Raij L. The pharmacokinetics and hemodynamics of Sildenafil Citrate in male

hemodialysis patients. Kidney International. 2004; 66: 367-374.
4. Cheng L, Dong HC, Jun SC. Effects of Efonidipine on the pharmacokinetics and pharmacodynamics of Eepaglinide: possible role of CYP3A4 and P-glycoprotein inhibition by Efonidipine. Journal of Pharmacokinetics and Pharmacodynamics. 2012; 39: 99-108.
5. Shinlapawittayatorn K, Chattipakorn S, Chattipakorn N. Effect of Sildenafil Citrate on the cardiovascular system. Brazilian Journal of Medical and Biological Research. 2005; 38: 1303-1311.
6. Kumari R, Kumar N, Hazra S, Paul KU, Bandyopadhyay A. Ocular Side Effects of Sildenafil.: A Prospective Study. International Journal of Scientific Study. 2005; 4: 7. https://doi.org/10.17354/ijss/2016/530.
7. Hyland R, Roe EGH, Jones BC, Smith DA. Identification of the cytochrome P450 enzymes involved in the N-demethylation of Sildenafil. British Journal of Clinical Pharmacology. 2001; 51: 239–248. https://doi.org/10.1046/j.1365-2125.2001.00318.x
8. Bae SH, Bae SK and Lee MG. Effect of hepatic CYP inhibitors on the metabolism of Sildenafil and formation of its metabolite, N-desmethylsildenafil, in rats in vitro and in vivo. Journal of Pharmacy and Pharmacology. 2009; 61: 1637-1642. https://doi.org/10.1211/jpp.61.12.0008.
9. Badyal DK, Dadhich AP. Cytochrome p450 and drug interactions. Indian Journal of Pharmacology. 2001; 33: 248-259.
10. Anzenbacher P, Anzenbacherova E. Cytochromes P450: Review on their Basic Principles. Indian National Science Academy. 2003; 69: 883- 991.
11. Horn JR, Hansten PD. Get to Know an Enzyme: CYP3A4. Drug Interactions, Insights & Observations. 2007; www.PharmacyTimes.com/Drug Interactions.
12. Grant RW. Cytochrome P4503A (CYP3A) metabolism: Prediction of In- Vivo activity in humans, Journal of Pharmacokinetics and Biopharmaceutics. 1996; 24: 475–490.
13. Xu Y, Zhang Y, Chen X, Zhong D. CYP3A4 inducer and inhibitor strongly affect the pharmacokinetics of triptolide and its derivative in rats. Acta Pharmacologica Sinica. 2017; https://doi.org/10.1038/aps.2017.170.
14. Watanabe A, Watari R, Ogawa K, Shimizu R, Tanaka Y, Takai N, Nezasa K, Yamaguchi Y. Using improved serial blood sampling method of mice to study pharmacokinetics and drug-drug interaction. Journal of Pharmaceutical Sciences. 2015; 104: 955-61. https://doi.org/10.1002/jps.24236.
15. Kurawattimath V, Pocha K, Mariappan TT, Trivedi RK, Mandlekar S. A modified serial blood sampling technique and utility of dried-blood spot technique in estimation of blood concentration: application in mouse pharmacokinetics, European Journal of Drug Metabolism and Pharmacokinetics. 2012; 37: 23-30. https://doi.org/10.1007/s13318-011-0066-5.
16. Lynch T, Price A. The Effect of Cytochrome P450 Metabolism on Drug Response, Interactions, and Adverse Effects Eastern Virginia Medical School. Norfolk, Virginia. 2007; 76: 391-6.
17. Buckle T, Ouwehand M, Beijnen JH, Schellens JHM, Tellingen OV, Bardelmeijer HA. Cannulation of the jugular vein in mice: a method for serial withdrawal of blood samples. Laboratory Animals. 2003; 37: 181–187. https://doi.org/10.1258/002367703766453010.
18. Uthayathas S, Karuppagounder SS, Thrash BM, Parameshwaran K, Suppiramaniam V, Dhanasekaran M. Versatile effects of Sildenafil: recent pharmacological applications. Pharmacological Reports. 2007; 59: 150–163.
19. Milman HA, Arnold SB. Neurologic, psychological, and aggressive disturbances with Sildenafil. Annals of Pharmacotherapy. 2002; 36:1129-34. https://doi.org/10.1345/aph.1A402
20. Krenzelok EP (2000) Sildenafil: clinical toxicology profile. Journal of Clinical Toxicology 38: 645-51.
21. Warrington JS, Shader RI, von Moltke LL, Greenblatt DJ. In vitro biotransformation of Sildenafil (Viagra): identification of human cytochromes and potential drug interactions. Drug Metabolism and Disposition. 2000; 28: 392-7.
22. Burgess G, Hoogkamer H, Collings L, Dingemanse J. Mutual pharmacokinetic interactions between steady-state bosentan and Sildenafil. European Journal of Clinical Pharmacology 64: 43-50.
23. Corbin JD, Francis SH. Pharmacology of phosphodiesterase-5 inhibitors. International Journal of Clinical Practice. 2002; 56: 453-9.
24. Jetter A, Kinzig-SM, Walchner-BM, Hering U, Bulitta J, Schreiner P, Sorgel F, Fuhr U. Effects of grapefruit juice on the pharmacokinetics of Sildenafil. Clinical Pharmacology & Therapeutics. 2002; 71: 21-29.
25. Mettu VS, Swami PY, P. Abigna, Nath AR, Sharma G. In Vitro and in Vivo (Mouse) Evaluation of Drug-Drug Interactions of Repaglinide with Anti-HIV Drugs, Pharmacology & Pharmacy. 2015; 6: 241-246.
26. Cooper JP, Hwang K, Singh H, Wang D, Reynolds CP, Curley RW, Williams SC, Maurer BJ and Kang MH. Fenretinide metabolism in humans and mice: utilizing pharmacological modulation of its metabolic pathway to increase systemic exposure. British Journal of Pharmacology. 2011; 163: 1263–1275, https://doi.org/10.1111/j.1476-5381.2011.01310.x.
27. Anderson JE, Blaschke TF. Ketoconazole Inhibits Cyclosporine Metabolism in Vivo in Mice. Journal of Pharmacology and Experimental Therapeutics. 1985; 236: 671- 674.
28. Lin YK, Sheu MT, Tzen TZ, Ho Ho. Biotransformation of Sildenafil in the male rat: evaluation of drug interactions with testosterone and Carbamazepine. Drug Development and Industrial Pharmacy. 2008; 34: 1219-26. https://doi.org/10.1080/03639040802005032.
29. Patel NJ, Wickremsinhe E, Hui Y, Barr A, Masterson N, Ruterbories K, Weller J, Hanes J, Kern T, Perkins E. Evaluation and Optimization of Blood Micro-Sampling Methods: Serial Sampling in a Cross-Over Design from an Individual Mouse. Journal of Pharmacy & Pharmaceutical Sciences. 2016; 19: 496 – 510.
30. Kurawattimath V, Pocha K, Mariappan TT, Trivedi RK, Mandlekar S. A modified serial blood sampling technique and utility of dried-blood spot technique in estimation of blood concentration: application in mouse pharmacokinetics.European Journal of Drug Metabolism and Pharmacokinetics. 2012; 37: 23–30. https://doi.org/10.1007/s13318-011-0066-5.
31. Joyce AP, Wang M, Lawrence-Henderson R, Filliettaz C, Leung SS, Xu X, O'Hara DM. One Mouse, One Pharmacokinetic Profile: Quantitative Whole Blood Serial Sampling for Biotherapeutics. Pharmaceutical Research. 2014; 31:1823-33. https://doi.org/10.1007/s11095-013-1286-y.
32. Zakari R, Allen KJ, Koplin JJ, Roche P, Greaves RF. Advantages and Challenges of Dried Blood Spot Analysis by Mass Spectrometry Across the Total Testing Process. The electronic Journal of the International Federation of Clinical Chemistry and Laboratory Medicine. 2016; 24: 288–317.