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Intestinal Absorption and Presystemic Disposition of Sildenafil Citrate in the Rabbit: Evidence for Site-dependent Absorptive Clearance

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ABSTRACT: Sildenafil citrate is the first oral treatment for erectile dysfunction. Its oral bioavailability is about 40%. This research investigated the intestinal transport parameters of sildenafil citrate in rabbit using an *in situ* intestinal perfusion technique. This was studied in four different anatomical sites, namely duodenum, jejunoileum, ascending colon and rectum. The results revealed the highest absorptive clearance in the jejunoileum. The values of the permeability area product normalized to segment length (ml/min.cm) were 0.0101, 0.0063, 0.0059 and 0.0023 and those of the percentage absorbed were 68.0, 32.3, 23.0 and 5.0 in jejunoileum, duodenum, ascending colon and rectum, respectively. The values of the length (cm) required for complete absorption were 87.6, 137, 153 and 384 for each anatomical site in the same order. The absorptive clearance did not correlate with the net water flux in the four anatomical regions studied, indicating a mainly passive diffusion mechanism through a transcellular pathway. The plasma sildenafil concentrations achieved during intestinal perfusion experiments and sildenafil total body clearance in the rabbit were used to calculate the fraction of sildenafil that reached the systemic circulation relative to the amount that disappeared from the intestinal segment. Only 34% of sildenafil that disappeared from the intestinal segment appeared in the systemic circulation indicating that the presystemic elimination of sildenafil is 66%. These results confirm that the incomplete bioavailability of sildenafil is mainly due presystemic elimination.

Key words: sildenafil absorption; sildenafil bioavailability; intestinal absorption; sildenafil

Introduction

Sildenafil citrate is a selective and potent inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type-5 (PDE-5), the predominant isoenzyme metabolizing cGMP in the corpus cavernosum. The inhibition of cGMP metabolism mediates the elevation of cGMP level after sexual stimulation, leading to corpus cavernosum smooth muscle relaxation, an increase in the penile blood flow and an increase in the intracavernosal pressure. All these effects result in an improvement in the erectile function. It has been confirmed that sildenafil citrate is an effective therapeutic agent for erectile dysfunction regardless of the etiology [1,2]. The oral absorption of sildenafil citrate is rapid when administered on an empty stomach, while food slows its absorption leading to a delayed onset of action. This was further confirmed with the rapid onset observed after sublingual administration when compared with oral administration. The absolute bioavailability of sildenafil after oral administration is only 38%–41% [3–5]. This incomplete bioavailability could be due to poor absorption from the gastrointestinal tract (GIT),

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improper targeting to the proper site of absorption in the GIT or due to presystemic elimination of sildenafil. The optimization of drug bioavailability in such cases requires the identification of the cause of the incomplete bioavailability.

The intestinal absorption of xenobiotics can be examined at different levels of integration, in the whole animal *in vivo*, in isolated intestinal segments *in situ* and in intestinal loops or even enterocytes *in vitro* [6]. The *in situ* methods bypass the effect of food, drug dissolution and stomach emptying steps after oral dosing while affording input control and a choice of the perfused intestinal segment. Also, the *in situ* methods provide intact lymph, blood and nerve supply for the solute uptake with extended tissue viability demonstrating superiority over *in vitro* methods. One of these techniques 'through-and-through' intestinal perfusion has been employed to monitor the absorption of several compounds, using rabbits as the model animal [7–9].

The primary objective of this research was to investigate the transport parameters of sildenafil citrate at the level of the intestinal membrane in the rabbit. This included qualitative and quantitative investigation of the transport mechanisms of sildenafil citrate across the intestinal membrane, which could be of significant importance in the design of dosage form and in optimizing the bioavailability of this drug. The membrane transport was studied at four different anatomical sites in the rabbit intestine, namely the duodenum, the jejunoileum, the ascending colon and the rectum utilizing the 'through-and-through' intestinal perfusion technique. In addition, the presystemic disposition of the drug was also calculated by measuring the concentration of sildenafil appearing in the plasma during the intestinal perfusion experiment. The rabbit was selected in this study as the model animal because the rabbit GIT physiology is similar to that of the human [10–12].

Experimental

Chemicals

Sildenafil citrate was kindly donated by MUP (Ismailia, Egypt), dexamethasone was obtained from Sigma Chemical Co. (St Louis, USA), monobasic sodium phosphate from Merck Chemical Co. (Darmstadt, Germany), acetonitrile HPLC-grade from BDH Laboratory (Poole, England), diethyl ether from Honil Ltd (London, UK), sodium chloride 0.9% (w/v) for injection USP, from El-Nasr Pharmaceutical Chemicals Company (Cairo, Egypt), ketamine HCl (100 mg/ml) from EIPICO Pharmaceuticals Company (10th of Ramadan City, Egypt), chlorpromazine HCl (25 mg/ml) from Misr Pharmaceuticals Company (Cairo, Egypt), pentobarbital sodium (50 mg/ml) from Abbott Laboratories (Chicago, USA), and Cal-Heparin ampoules 5000 IU/ml, Amoun Pharmaceutical Co. (Al-Obour City, Egypt).

Isolated intestinal segments preparation

The procedures for the preparation of the isolated intestinal segments for the *in situ* perfusion experiment were described in detail previously [7]. Male albino rabbits weighing 2.8–3.1 kg were used in these experiments. After an overnight fast, the rabbits were anesthetized by intramuscular (i.m.) injections of ketamine HCl (two doses of 45 mg/kg at 15 min intervals and an additional dose of 25 mg/kg if needed). Chloropromazine HCl was used as a muscle relaxant and was given before each anesthetic dose. The dose of the muscle relaxant was 2 mg/kg given i.m.

After induction of anesthesia, the rabbit was laid in a supine position on a heating pad and the abdominal area was shaved and cleaned with a depilatory cream. A longitudinal incision 6–8 cm was made, and the intestinal segments of interest were exposed and isolated carefully. In order to cannulate the jejunoileum segment, the proximal end (60 cm from the pylorus) was tied off using surgical thread, and was cannulated using a 3-way stopcock cannula. The desired length of the intestinal segment was then measured and the distal end was cannulated using an L-shaped glass cannula. A length of 30 cm was used throughout the study except in the group which was used to correlate the intestinal absorption with the plasma concentration where a 60 cm segment was used. The use of a longer segment was to increase the amount of sildenafil absorbed

and to produce higher sildenafil concentrations in the plasma.

For the colon, the proximal end was tied off immediately after the ampulla coli, the desired length (15 cm) was measured, and finally the distal end was tied off. Two incisions were made, one on each end, and the solid fecal debris were squeezed out by gentle manipulation of the segment, and by infusing normal saline through the proximal end. Finally, both the proximal and distal ends were cannulated as described before. The same procedures were applied to both the duodenum and rectum taking the length of the duodenum as 15 cm and that of the rectum as 5 cm. Each rabbit was utilized to study the absorption from two different intestinal segments at the same time.

The intestinal segment under study was carefully arranged in a uniform S- to multi-S-pattern, to avoid kinks and to ensure uniformity in the intestinal fluid flow during perfusion. The isolated segment was kept warm and moist by frequent application of 37 °C normal saline to a gauze pad covering the intestine. The remainder of the intestine was returned into the abdominal cavity for better maintenance of the temperature. At the end of the experiment the animal was killed by injecting an overdose of pentobarbital sodium through the marginal ear vein. The intestinal segments under study were excised and were accurately measured.

Anatomical difference in sildenafil absorption

The first objective of this research was to characterize the anatomical difference in the absorption of sildenafil citrate from four different anatomical sites of the rabbit GIT: duodenum, jejunoileum, colon and rectum. For this purpose, six animals divided into two groups ($n = 3$) were used. The first group was utilized to study the absorption from the duodenum and the rectum, while the second group was used to study the absorption from the jejunoileum and the ascending colon.

The perfusion solution (13.6 μg/ml sildenafil citrate) was prepared in 0.9% (w/v) sodium chloride for injection, USP. After surgical preparation of the rabbits, sildenafil citrate solution was perfused into the intestinal segment under investigation at a flow rate of 0.27 ml/min using a constant rate perfusion pump (Harvard-22, Harvard Apparatus, Millis, MA, USA). The effluent from the perfused segment was collected at 10 min intervals for 120 min in 10 ml preweighed stoppered tubes. These tubes were weighed again after sample collection, and the effluent weight was recorded as the difference.

The net water flux which results from the transmucosal fluid movement, was determined gravimetrically from the difference of the volume of the intestinal perfusion rate and the volume of the intestinal segment outflow taking the density of the aqueous samples as unity. The concentration of the drug in the effluent was corrected accordingly.

Presystemic disposition of sildenafil absorption

The second objective of this research was to estimate the presystemic disposition of sildenafil. For this purpose, two groups of rabbits ($n = 3$ each) were used. The rabbits of the first group were prepared as described above for perfusion of a 60 cm segment of the jejunoileum. The jejunoileum segment was selected in this experiment because the regional absorption studies showed that this segment has the highest sildenafil absorption. Also, the marginal ear vein was cannulated using an i.v.-placement catheter (Charter Med. Inc., NJ, USA). Blood samples (1 ml) were collected every 10 min during the intestinal perfusion and throughout the experiment. The blood samples were collected in heparinized tubes. These tubes were pretreated with one drop of heparin 5000 U/ml the day before the experiment and were left to dry at room temperature. Plasma was separated by centrifugation at 1000 *g* for 10 min and was frozen at −20 °C until analysis.

The steady state sildenafil concentration achieved during the jejunoileum perfusion is dependent on the total body clearance of sildenafil in the rabbit and the amount of sildenafil that reaches the systemic circulation during the jejunoileum perfusion. Therefore, it was necessary to estimate the total body clearance of sildenafil in the rabbits in order to calculate the fraction of the absorbed sildenafil that reached the systemic circulation. For this purpose, a

second group of rabbits ($n = 3$) was used to calculate the total body clearance of sildenafil. The rabbits were anesthetized as described above and both marginal ear veins were cannulated. One catheter was used to infuse 110 μg/ml sildenafil citrate at a rate of 0.1 ml/min after an i.v. bolus dose of 1 mg sildenafil, and the second catheter was used to collect blood samples. Blood samples (1 ml) were collected every 30 min for a period of 5 h. Plasma was separated by centrifugation and was frozen at −20 °C until analysis.

Sample analysis

The perfusate samples collected during the intestinal perfusion were centrifuged at 1000 *g* for 5 min in order to precipitate any mucus debris. A set of clean test tubes was spiked with 100 μl of the internal standard methanol solution (dexamethasone, 100 μg/ml), and the tubes were left to dry overnight. An aliquot of 0.5 ml from each perfusate sample, and 0.5 ml of the mobile phase were transferred to the test tubes spiked with the internal standard. The final dexamethasone concentration was 10 μg/ml. Thirty microliters of the resulting solution were injected onto the HPLC. Standard sildenafil solutions were prepared by addition of 1 ml mobile phase to a series of test tubes spiked with the internal standard to produce a final concentration of 10 μg/ml, and sildenafil citrate sufficient to produce a concentration range 0.1–20 μg/ml.

Rabbit plasma samples were analysed by transferring 0.5 ml of rabbit plasma to clean test tubes spiked with the internal standard to produce a dexamethasone concentration of 2 μg/ml. The resulting solution was extracted with 4 ml of diethyl ether for 5 min, and then centrifuged at 1000 *g* for 5 min. The ether layer was transferred to clean test tubes and was evaporated in a water bath at 45 °C. The residue was reconstituted in 250 μl of the mobile phase and 30 μl of the resulting solution were injected onto the HPLC. Standard sildenafil samples were prepared similarly by extracting blank rabbit plasma samples spiked with known sildenafil concentration in the range 0.05–5 μg/ml. Calibration curves were constructed from the obtained peak area ratio (drug peak area/internal standard peak area) and the concentration of sildenafil in each standard sample. The concentrations of sildenafil in the unknown samples were determined from the regression equation of the calibration curves.

A high pressure liquid chromatograph (Waters^{TM} 600 controller, Waters, USA) equipped with a variable wavelength detector (Waters^{TM} 486) and an automatic sampling system (Waters^{TM} 717) was used for the analysis of the samples. The mobile phase consisted of 20 mM monobasic sodium phosphate:acetonitrile (65:35), and the flow rate was 1.3 ml/min. Separation was achieved using a 15 cm × 3.9 mm (i.d.) C_{18}, μ Bondapak^{TM}, Waters, reversed phase column with an average particle size of 10 μm, and the column was kept at ambient temperature. The column effluent was monitored at 240 nm and the chromatographic data analysis was performed with the Millenium^{TM} Program (Waters, USA). The assay was fully validated for selectivity, linearity, precision, accuracy and stability using blank human plasma. The coefficient of variation for the within-day precision determined from the analysis of three calibration curves on the same day and the between-day precision determined from the analysis of six calibration curves on 6 different days was in the range 1%–9%, while the accuracy of the analysis of sildenafil standards was always within 10% of the nominal concentrations.

Data analysis

Absorptive clearance. The flow rate was estimated for each perfusion experiment from the linear regression of the volume remaining in the perfusion syringe versus time. The volume of the outflow samples was estimated gravimetrically taking the density of the aqueous samples as 1 (the same as water) [7–9]. From the difference in the flow rate entering and leaving the intestinal segment, the outflow concentration was corrected for the net water flux. The ratio between the corrected concentration at the outflow $\{C_{(out)}\}$ and that at the inflow $\{C_{(in)}\}$ was calculated for each perfusate sample collected. The average of the outflow-to-inflow concentration ratios for the fractions collected from 70 to 120 min was taken as the steady-state ratio. This

ratio at steady-state is given by [13–17]

$$\{C_{(out)}/C_{(in)}\}_{ss} = \exp^{-(PeA/Q)} \quad (1)$$

where A is the effective surface area (cm^2), Pe is the apparent permeability coefficient (cm/min), and Q is the average flow rate within the intestinal segment (ml/min). Rearrangement of Equation (1) allows the permeability-area product (PeA) to be calculated

$$PeA = -Q.\ln(C_{(out)}/C_{(in)})_{ss} \quad (2)$$

When employing the *in situ* intestinal perfusion technique, the term (PeA) should be normalized to the length of the intestinal segment in order to allow for comparison of the effective permeability of segments having different lengths.

Since $\{C_{(out)}/(C_{(in)})\}_{ss}$ is the fraction remaining after the solution has passed through the intestinal length, then the fraction absorbed is

$$Fa = 1 - \{(C_{(out)})/(C_{(in)})\}_{ss} = 1 - \exp^{-(PeA/Q)} \quad (3)$$

Associated with the concept of intestinal absorption is the reserve length [15]. The anatomical reserve length (ARL), is defined as the length of the intestine remaining after absorption has been completed, and it is given by:

$$ARL = (L^*) - (l^*) \quad (4)$$

where ARL is the anatomical reserve length (cm), L^* is the maximal intestinal length available for absorption (cm), and l^* is the intestinal length along which absorption is complete (cm). A negative value for the ARL indicates that the absorption of the drug is not complete in this anatomical segment. In theory, the bulk luminal concentration will never be reduced to zero at the intestinal length (l^*), due to the nature of the logarithmic function. Accordingly, an arbitrary small fraction of solute remaining will be considered as the criteria for complete absorption. Taking this fraction as 5%, and substituting in Equation (1) will give the following equation:

$$0.05 = \exp^{-\{(PeA.l^*)/Q\}} \quad (5)$$

where PeA is the effective permeability surface area product normalized to length, and l^* is the length required for 95% absorption ($L95\%$) of a given solute.

Effect of solvent drag on intestinal absorptive clearance. The net amount of drug absorbed per unit time can be described as the sum of two terms; the diffusive contribution and the convective contribution corresponding to the solvent drag effect. The net amount of drug absorbed per unit time is then given by the following equation [18–20].

$$Js = Ks(C - C_p) + \varnothing s J_w C \quad (6)$$

In which the first term on the right is diffusive and the second is convective and Js is the rate of absorption of the solute from the lumen (μg/min) and is given by $\Delta Ns/\Delta t$ where, ΔNs is the amount of the solute (μg) absorbed in a time interval Δt (min). Ks is the diffusive permeability coefficient and is given by $DAKp/\Delta x$, in which D is the diffusion coefficient of the solute, A is the effective surface area, Kp is the (octanol/water) partition coefficient of the compound, and Δx is the path length. C and C_p are the solute concentrations in the lumen and plasma, respectively. $\varnothing s$, is the sieving coefficient of the given compound; it represents the ratio between the concentration of the compound in the convective stream and that in the luminal fluid. $\varnothing s$ equals $1–\sigma$, where σ is the Staverman reflection coefficient of a given compound and represents its interaction with water. Jw is the rate of fluid (or water) flux without reference to its mechanism (osmotic, hydrostatic or electrical). The water flux is an absorption-secretion process which is dependent on the experimental parameters during the perfusion. Jw, Ks and $\varnothing s$ are assumed to remain constant during a given experimental run. At the steady state, due to sink conditions in the blood, Equation (6) is reduced to

$$J_{ss} = DAKp/\Delta x(C_{ss}) + \varnothing Jw(C_{ss}) \quad (7)$$

where J_{ss} is the steady state solute flux (μg/min) and C_{ss} is the length averaged steady state concentration of the solute in the lumen (μg/ml).

Rearrangement of Equation (7) gives the following equation.

$$J_{ss}/C_{ss} = DAKp/\Delta x + \varnothing Jw \quad (8)$$

The term J_{ss}/C_{ss} represents the overall absorptive clearance of the given solute (ml/min), regardless its route or mechanism, and practically it is estimated as the overall absorptive

clearance (*PeA*) (Equation (2)). When the absorptive clearance is correlated with the net water flux, this indicates that the absorption of the drug is dependent on the water flux and the absorption mechanisms include a paracellular component. On the other hand if the permeability coefficient term ($DAKp/\Delta x$) is different from zero, this indicates transcellular drug absorption.

Calculation of the presystemic elimination of sildenafil. The steady state plasma concentration achieved during the intestinal perfusion experiment can be expressed by the following term,

$$Cp_{ss} = \frac{FR}{CL_{tot}} \tag{9}$$

where Cp_{ss} is the steady state plasma concentration, F is the fraction of the drug which reaches the systemic circulation, R is the amount of the drug that disappears during the intestinal perfusion and is equal to ($R_{in}-R_{out}$), while CL_{tot} is the total body clearance of the drug. CL_{tot} is calculated during the i.v. infusion of the drug from the infusion rate (K_o), and the steady state plasma concentration during the infusion (Cp_{ss})

$$Cp_{ss} = \frac{K_o}{CL_{tot}} \tag{10}$$

F can be calculated from Equation (9) by substituting for the calculated values for R, CL_{tot} and plasma sildenafil concentration achieved during the intestinal perfusion experiment.

Results

The extent of sildenafil absorption was different in the different intestinal segments investigated in this study. The fraction of sildenafil absorbed during the perfusion of different anatomical segments of the rabbit intestine is presented in Figure 1. The absorptive clearance of sildenafil citrate normalized to the intestinal length (*PeA/L*) calculated for the different anatomical regions (Table 1) showed significant differences among the studied segments. The absorption of sildenafil was in the order of jejunoileum > duodenum > ascending colon > rectum, which is also clear in Figure 1. These results indicate differences in sildenafil permeability in the different intestinal regions. The calculated values for the mean ± standard error for absorptive clearance normalized for the intestinal length were 0.0101 ± 0.0028, 0.00633 ± 0.0006, 0.0059 ± 0.0006 and 0.00232 ± 0.0003 ml/min.cm for the jejunoileum, duodenum, ascending colon and rectum, respectively. The length required for complete absorption of sildenafil in the different intestinal segments (*L*95%) and the anatomical reserve length were calculated and presented in Table 1. The negative *ARL*s for the duodenum, ascending colon and the rectum indicate that sildenafil cannot be absorbed completely from these anatomical segments.

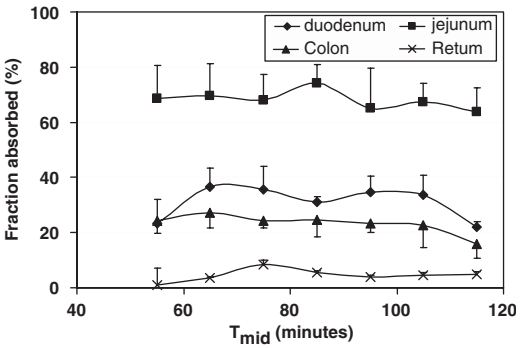

Figure 1. The fraction of sildenafil absorbed during the perfusion of different anatomical segments of the rabbit intestine. (Data at steady state are presented as mean ± SEM, $n = 3$)

The effect of net water flux on the absorptive clearance of sildenafil was studied according to Lifson's model [18]. Figure 2 represents a plot of sildenafil absorptive clearance versus the net water flux both normalized to the segment length at steady state for the different intestinal segments. The regression parameters for the different intestinal segments are presented in the legends of Figure 2. The slopes of the plot for the different segments were not significantly different from zero indicating the independence of the absorptive clearance of sildenafil on the water flux, and the absence of paracellular absorption. While the intercepts of the plots were significantly different from zero suggesting mainly transcellular absorption.

The average plasma sildenafil concentration achieved after 6 h of a constant rate infusion of

Table 1. Membrane transport parameters of sildenafil in the four different GIT segments

Parameter	Duodenum	Jejunoileum	Ascending colon	Rectum
Segment length (cm)	15	30	15	5
PeA (ml/min)	0.104	0.321	0.067	0.013
	(0.0138)	(0.0917)	(0.0083)	(0.0019)
R_{out}/R_{in}	0.677	0.320	0.770	0.95
	(0.03)	(0.09)	(0.03)	(0.01)
% Fa	32.3	68.0	23.0	5.0
	(3.25)	(9.28)	(2.54)	(0.68)
PeA/L	0.00633	0.0101	0.0059	0.00232
(ml/min.cm)	(0.0006)	(0.0028)	(0.0006)	(0.0003)
l^* (*L*95%)	137	87.6	153	384
(cm)	(10.1)	(19.97)	(18.75)	(41.44)
ARL (cm)	−117	32.4	−138	−379
	(10.2)	(20.0)	(18.8)	(41.4)
JW	0.0296	0.0346	0.0387	0.0332
(ml/min)	(0.0014)	(0.0032)	(0.0057)	(0.0129)
JW/L	0.0018	0.0011	0.0034	0.0057
(ml/min.cm)	(0.0001)	(0.0002)	(0.0004)	(0.0020)

PeA is the overall absorptive clearance, R_{out}/R_{in} is the fraction remaining to be absorbed, *%Fa* is the percentage fraction absorbed, *PeA/L* is the effective permeability surface area product normalized to the segment length, *L*95% is the length required for 95% absorption, *ARL*s is the anatomical reserve length which is the difference between the total length of the total anatomical segment and the *L*95%, *JW* is the water flux and *JW/L* is the water flux normalized to the segment length. Values between brackets are the standard error, $n = 3$.

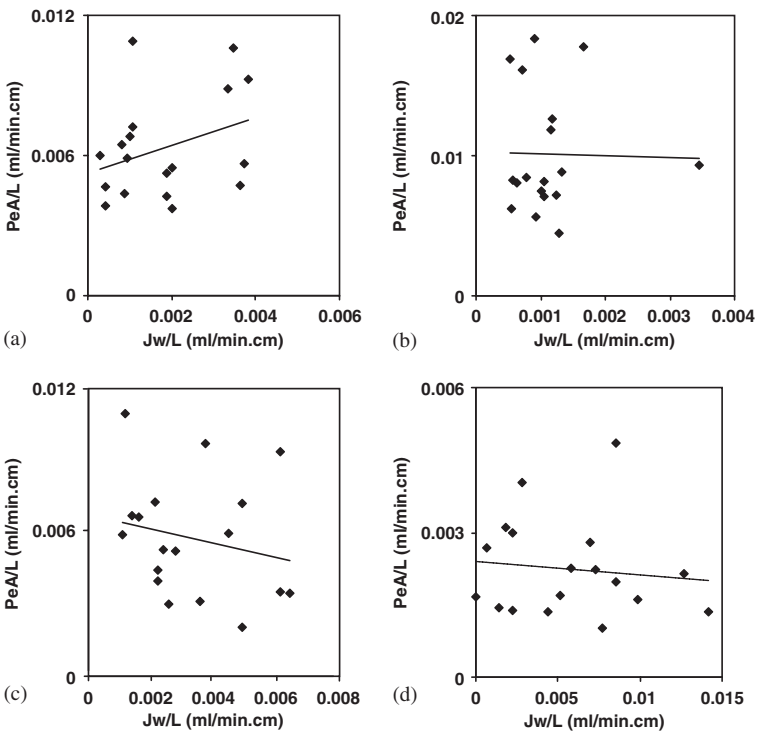

Figure 2. Absorptive clearance of sildenafil versus water flux in different intestinal segments; Parameters are normalized to segment length. (a) duodenum, Line equation: $y = 0.00526(0.00091) + 0.5857(0.4173)x$; (b) jejunoileum, Line equation: $y = 0.0103(0.0021) - 0.128(0.1663)x$; (c) colon, Line equation: $y = 0.00694(0.00131) - 0.301(0.343)x$; (d) rectum, Line equation: $y = 0.00249(0.00044) - 0.0291(0.0633)x$. Values in parentheses are the standard error values, $n = 3$. The intercept ($DAKp/\Delta x$) is the permeability coefficient and the slope ($\varnothing s$) is the sieving coefficient. All the slopes are not significantly different from zero ($p > 0.05$), and all the intercept values are significantly different from zero ($p < 0.01$)

sildenafil of 11 μg/min was 0.626 ± 0.21 μg/ml. The differences in the last three sildenafil concentrations were less than 10% indicating that a steady state was achieved. The average total body clearance calculated from the sildenafil infusion rate and the average steady state concentration was 17.57 ml/min. This average total body clearance was used for the calculation of the presystemic elimination parameters for sildenafil during the intestinal perfusion experiment.

The plasma sildenafil concentration achieved at the end of the intestinal perfusion experiment was calculated from the average value of the last three concentrations. The last three concentrations were always within 10% indicating that these concentrations can be a good approximation of the steady state concentration. The bioavailability of sildenafil during the intestinal perfusion experiment was calculated in each rabbit from the rate of drug disappearance from the perfused segment, the plasma concentration and the average estimate for sildenafil total body clearance. The calculated parameters for sildenafil presystemic elimination during the intestinal perfusion experiment are summarized in Table 2.

Discussion

The oral bioavailability of sildenafil in humans has been reported to be 38%–41% [3–5]. It is important to investigate the cause of this incomplete bioavailability in order to determine the factors that may alter the drug bioavailability and hence its pharmacological effects. Also, proper design of the dosage form in order to optimize drug absorption and bioavailability depends on understanding the membrane transport characteristics and the mechanisms of drug absorption.

The absorption of sildenafil is different in the four different GIT segments investigated in the current study with the highest absorption occurring from the jejunoileum followed by the duodenum, the ascending colon and the rectum, respectively. This was apparent from the different fraction of the drug absorbed during the perfusion experiments, the absorptive clearance normalized for the segment length, and the anatomical reserve length (Table 1). These results may suggest that the absorption of sildenafil occurs mainly from the jejunoileum and that slowing the gastric emptying rate may lead to slowing sildenafil absorption. It should be noted that the length of jejunoileum required for 95% absorption was only 87.58 cm. This indicates that the oral absorption of sildenafil is nearly complete.

The poor correlation between the absorptive clearance of sildenafil and the net water flux in the different GIT segments investigated in this study indicates that its absorption is primarily through the transcellular pathway. This is apparent from the slope and the intercept of the regression between the absorptive clearance and the net water flux. These results can explain the difference in sildenafil absorption from the different GIT segments, which have different surface areas per unit length. The extent of sildenafil absorption from the different anatomical segments is in the same order of the surface area per unit length with the jejunoileum showing the highest and the rectum showing the lowest absorption.

Table 2. Sildenafil pharmacokinetic parameter and presystemic elimination parameters

Parameter	Rabbit 1	Rabbit 2	Rabbit 3	Mean	SD
Sildenafil perfusion rate (R_{in}, μg/min)		3.672			
Amount of sildenafil disappeared during the intestinal perfusion ($R_{in}-R_{out}$, μg/min)	3.345	3.275	3.460	3.360	0.093
Average plasma concentration achieved during the intestinal perfusion experiment (μg/ml)	0.0514	0.0987	0.0453	0.0651	0.029
Fraction of sildenafil that reaches the systemic circulation during the intestinal perfusion[a]	0.27	0.53	0.23	0.343	0.163
Presystemic elimination	73%	47%	77%	65.7	16.3

[a] This was calculated from Equation (9), taking the average value of CL_{tot} as 17.57 ml/min.

The results of this investigation clearly indicate complete absorption of sildenafil from the GIT. This excludes any role for the membrane transport in the reported poor bioavailability of sildenafil. Accordingly, the presystemic disposition of sildenafil was considered. Our investigation showed that about 66% of the absorbed drug was eliminated before reaching the systemic circulation. This presystemic elimination resulted in only 34% of the absorbed drug being bioavailable. The primary cytochrome P450 isoform responsible for sildenafil metabolism is mainly CYP3A4, with CYP2C9 playing a minor role [21]. These CYP450 isoforms are present in large amounts in the intestine and the liver and are responsible for the extensive presystemic elimination of sildenafil. The clinical significance of this is that administration of sildenafil with inhibitors of these CYP450 isoforms such as cimetidine, macrolide antibiotics and protease inhibitors, can significantly increase sildenafil bioavailability which can lead to augmentation of its pharmacological and adverse effects [22,23].

The results indicated that improved sildenafil bioavailability after oral administration cannot be achieved by enhancing drug absorption because its absorption is almost complete from the GIT. The improved bioavailability can be obtained by avoiding the presystemic metabolism. This can be achieved for example through the rectal route. However, our study revealed poor rectal absorption of sildenafil. Thus if a rectal dosage form is to be designed an absorption enhancer must be included. The results of our study suggested mainly transcellular absorption of the drug from all segments. Thus the absorption enhancer must be selected among those acting on the transcellular mechanism (such as bile salts) and those influencing the paracellular route must be excluded.

Conclusions

The intestinal absorption of sildenafil citrate was site dependent with the absorption in the order of jejunoileum > duodenum > ascending colon > rectum. The study revealed apparently complete absorption from GIT with the absorption being mainly transcellular and with no evidence for a paracellular mechanism in any segment.

Presystemic elimination was mainly responsible for the poor bioavailability of sildenafil. Accordingly, avoidance of the presystemic metabolism is the recommended approach to increase sildenafil bioavailability. Finally, if a rectal dosage form is to be designed, an absorption enhancer must be included. This enhancer must improve transcellular absorption which is the major pathway for sildenafil permeation.

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