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ORIGINAL ARTICLE

The utility of rat jejunal permeability for biopharmaceutics classification system

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Abstract

Purpose: The biopharmaceutical classification system has been developed to provide a scientific approach for classifying drug compounds based on their dose/solubility ratio and human intestinal permeability. Therefore in this study a new classification is presented, which is based on a correlation between rat and human intestinal permeability values. *Methods*: In situ technique in rat jejunum was used to determine the effective intestinal permeability of tested drugs. Then three dimensionless parameters—dose number, absorption number, and dissolution number (D_o, A_n, and D_n)—were calculated for each drug. *Results*: Four classes of drugs were defined, that is, class I, $D_0 < 0.5$, $P_{eff(rat)} > 5.09 \times 10^{-5}$ cm/s; class II, $D_o > 1$, $P_{eff(rat)} > 5.09 \times 10^{-5}$ cm/s; class III, $D_0 < 0.5$, $P_{eff(rat)} < 4.2 \times 10^{-5}$ cm/s; and class IV, $D_o > 1$, $P_{eff(rat)} < 4.2 \times 10^{-5}$ cm/s. A region of borderline drugs ($0.5 < D_o < 1$, $4.2 \times 10^{-5} < P_{eff(rat)} < 5.09 \times 10^{-5}$ cm/s) was also defined. *Conclusion*: According to obtained results and proposed classification for drugs, it is concluded that drugs could be categorized correctly based on dose number and their intestinal permeability values in rat model using single-pass intestinal perfusion technique. This classification enables us to remark defined characteristics for intestinal absorption of all four classes using suitable cutoff points for both dose number and rat effective intestinal permeability values.

Key words: *Biopharmaceutics classification; dissolution; permeability; rat jejunal permeability; solubility; single-pass intestinal perfusion*

Introduction

In 1995 Amidon et al. devised a biopharmaceutics classification system (BCS) to classify drugs based on their aqueous solubility and intestinal permeability, two fundamental properties governing drug absorption[1]. This system divides active moieties into four classes: class I (high permeability and high solubility), class II (high permeability and low solubility), class III (low permeability and high solubility), and class IV (low permeability and low solubility). For highly permeable drugs the extent of fraction dose absorbed in humans is considered to be more than 90% as defined by US Food and Drug Administration[2,3]. The classification of drug solubility is based on the dimensionless dose number (D_o), which is the ratio of drug concentration in the administered volume (250 mL) to the saturation solubility of the drug in water. If a drug has dose/solubility ratio less than 250 mL over the pH range from 1 to 7.5 it is classified as a highly soluble drug compound[4]. BCS classification can help pharmaceutical companies to save a significant amount in development time and to reduce costs. This classification provides a regulatory tool to substitute in vivo bioequivalence (BE) studies by in vitro dissolution tests. In fact for immediate-release solid oral dosage forms containing rapidly dissolving and easily permeating active ingredients BE studies may not be required because they act like a solution after oral administration. Therefore

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dissolution rate has a negligible impact on bioavailability of highly soluble and highly permeable (BCS class I) drugs. As a result, various regulatory agencies including the US Food and Drug Administration now allow BE of formulations of BCS class I drugs to be demonstrated by in vitro dissolution (often called a biowaiver)[5]. Waivers for class III drugs have also been recommended[6,7]. Moreover, BCS provides distinct rules for determining the rate-limiting factor in the gastrointestinal drug absorption process. As a result it could be helpful in the selection of candidate drugs for full development, prediction and clarification of food interactions, choice of formulation principle, and the possibility of in vitro–in vivo correlation in the dissolution testing of solid formulations[2,8]. Three groups of methods have emerged to determine the intestinal absorptive potential of a drug and the mechanism of absorption. These groups include in vivo, in situ, and in vitro methods[9,10]. Among these methods single-pass intestinal perfusion approach is the most frequently used technique that provides conditions closer to what is faced following oral administration[11]. The suitability of this method to predict human intestinal permeability and fraction dose absorbed was established in a previous paper using a sufficient number of model dugs[11]. Although permeability classification of drugs would be ideally based on human jejunal permeability data, such information is available for only a small number of drugs. Therefore, in this study a new classification is presented, which is based on a correlation between rat and human intestinal permeability values.

Materials and methods

Chemicals

Naproxen and ketoprofen were provided by Sigma (St. Louis, MO, USA) and Wako (Osaka, Japan), respectively. Furosemide and hydrochlorothiazide were purchased from Shasun Chemicals and Drugs Ltd. (Pondicherry, India). Propranolol was obtained from ICI-Pharma (Madrid, Spain) and metoprolol was from Ciba-Geigy (Barcelona, Spain). Phenol red was purchased from Sigma. Acetonitrile and methanol were of high-performance liquid chromatography (HPLC) grade and obtained from Merck (Darmstadt, Germany). KH_2PO_4, NaH_2PO_4, Na_2HPO_4, orthophosphoric acid, NaOH, NaCl, glacial acetic acid, and triethylamine were purchased from Merck as well. Double-distilled water was used during the entire HPLC procedure.

HPLC analysis of test drugs

All samples were analyzed by reverse-phase HPLC. For samples containing naproxen and ketoprofen the mobile phase was a mixture of 19.9% methanol, 27.9% of acetonitrile, 51.8% water, and 0.4% triethylamine (adjusted to pH 3.2) [limit of quantification (LOQ) = 0.3 and 0.25 ng/mL for naproxen and ketoprofen, respectively][12]. The mobile phase for furosemide, antipyrine, and hydrochlorothiazide samples consisted of 42% acetonitrile, 58% water, 0.9% glacial acetic acid, and 0.1% triethylamine (adjusted to pH 5.6) (LOQ = 0.72, 100, and 0.9 ng/mL for furosemide, antipyrine, and hydrochlorothiazide, respectively)[13]. Metoprolol and propranolol were analyzed using 55% methanol, 45% of 0.05 M KH_2PO_4 aqueous solution (adjusted to pH 6), and 0.2% triethylamine as mobile phase (LOQ = 14 and 7.2 ng/mL for metoprolol and propranolol, respectively)[14]. Detection wavelengths were 270, 280, and 227 nm, respectively. For other drugs the composition of mobile phases and detection wavelengths were as follows—piroxicam: 39% acetonitrile, 61% sodium acetate 0.1 M, and 0.05% triethylamine (adjusted to pH 2.6), λ = 330 nm (LOQ = 60 ng/mL)[15]; atenolol: 10% acetonitrile, 90% phosphate buffer 0.67 (pH = 7.4), and 0.2% triethylamine (adjusted to pH 3), λ = 225 nm (LOQ = 195 ng/mL)[16]; cimetidine and ranitidine: 78% KH_2PO_4 0.05 M, 22% acetonitrile, and 0.05% triethylamine (adjusted to pH 8), λ = 229 nm (LOQ = 28 and 42 ng/mL for cimetidine and ranitidine, respectively); carbamazepine: 67% methanol, 33% water, and 1% glacial acetic acid, λ = 230 nm (LOQ = 15 ng/mL)[17]; phenol red: 45% KH_2PO_4 0.05 M and 55% methanol (adjusted to pH 2.6), λ = 430 nm (LOQ = 20 ng/mL)[18]; and ibuprofen: 85% acetonitrile, 15% of 0.067 M phosphate buffer, and 0.2% mL orthophosphoric acid, λ = 254 nm (LOQ = 24 ng/mL)[19]. The mobile phases were filtered through sintered glass filter *P*5 (1–1.6 micron) (Winteg, Hattert, Germany) and degassed in sonicator (Liarre, Bologna, Italy) under vacuum and then were pumped in isocratic mode in all cases.

In situ permeation studies

In situ permeation studies were performed using established methods adapted from the literature[20,21]. Briefly, male Wistar rats (weight, 250–300 g; age, 7–9 weeks) were maintained on 12 hours light–dark cycle and fasted 12–18 hours before experiment. However, drinking water was readily accessible. The rats were anesthetized using an intraperitoneal injection of pentobarbital (60 mg/kg) and were placed on a heated pad to keep normal body temperature. By making a midline abdominal incision, a 10-cm section of the proximal rat jejunum was located and gently rinsed with saline (37°C) and attached to the perfusion assembly. Care was taken to handle the small intestine gently and to minimize the surgery to maintain intact blood supply. The entire surgical area was then covered with parafilm to reduce evaporation. Blank perfusion buffer was infused for 10 minutes by a syringe pump (Palmer, Surrey, UK) followed by perfusion of

compounds at a flow rate of 0.2 mL/min for 90 minutes. The outlet perfusate was collected every 10 minutes in microtubes. The length of segment was measured at the end, and finally the animal was killed with a cardiac injection of saturated solution of KCl. Samples were stored at −20°C until analysis. In all animal studies 'Guide to the care and use of experimental animals', by Canadian Council on Animal Care, was followed[22]. Permeability values were calculated using following equation according to the parallel tube model[23,24]:

$$P_{\text{eff}} = \frac{-Q\ln(C_{\text{out(corrected)}}/C_{\text{in}})}{2\pi rl},$$

where C_{in} is the inlet concentration and C_{out} is outlet concentration of compound, which is corrected for volume change in segment using phenol red concentration in inlet and outlet tubing. Q is the flow rate (0.2 mL/min), l is the length of the intestinal segment, and r is the rat intestinal radius (0.18 cm)[23].

Dose number calculation

Dose number is a criterion for solubility (D_{o}), which is defined as the ratio of dose concentration to drug solubility[25]. It is calculated as follows:

$$D_{\text{o}} = \frac{M/V_{\text{o}}}{C_{\text{s}}},$$

where C_{s} is the solubility, M is the maximum dose strength, and V_{o} is the volume of water taken with the dose (generally set to be 250 mL). The values of solubility and maximum dose strength of tested compounds are listed in Table 1[4]. Dose number would be as unity ($D_{\text{o}} = 1$), when the maximum dose strength is soluble in 250 mL of water and the drug is in solution form throughout the gastrointestinal tract. This criterion is extended to 0.5 for borderline classification, considering the average volume of fluid (500 mL) under fed conditions.

Dissolution number calculation

Dissolution number refers to the time required for drug dissolution, which is the ratio of the intestinal residence time to the dissolution time, which includes solubility (C_{s}), diffusivity (D), density (ρ), initial particle radius (r_0) of a compound, and the intestinal transit time (T_{si})[26].

$$D_{\text{n}} = \left(\frac{3D}{r_0^2}\right)\left(\frac{C_{\text{s}}}{\rho}\right)\langle T_{\text{si}}\rangle = \frac{\langle T_{\text{si}}\rangle}{\langle T_{\text{diss}}\rangle},$$

where ρ and T_{si} are generally considered to be 1200 mg/cm^3 and 199 minutes, respectively.

$$T_{\text{diss}} = \frac{\rho h r_0}{3DC_{\text{s}}}.$$

Absorption number calculation

This is the ratio of permeability (P_{eff}) and the gut radius (R) times the residence time in the small intestine,

Table 1. Dose, solubility, and calculated oral drug absorption parameters for tested compounds.

Compound	Mean P_{eff} (10^5 cm/s)	Dose (mg)[a]	C_{s} (mg/mL)	D_{o} calculated	A_n calculated	D_n calculated	T_{abs} calculated (min)	T_{diss} calculated (min)	D_{abs} calculated (mg)
Antipyrine	5.9 ± 0.2	250	1000[a]	0.001	2.58	11,784.9	76.5	0.01	3,519,359
Propranolol	5.6 ± 2.0	90	33[a]	0.011	2.44	302.1	81.1	0.65	109,621
Carbamazepine	6.2 ± 0.6	200	0.01[a]	80.0	2.78	0.10	71.2	1915.2	38
Ibuprofen	20 ± 2.2	400	0.01[a]	160.0	10.99	0.08	18.0	2249.7	150
Ketoprofen	9.6 ± 1.8	50	0.05[b]	4.0	4.81	0.50	41.1	395.8	328
Naproxen	11 ± 0.2	500	0.01[b]	200.0	5.98	0.10	33.1	1947.3	81
Piroxicam	7.9 ± 4.0	10	0.005[a]	8.0	3.79	0.04	52.1	4204.7	26
Metoprolol	3.3 ± 1.5	100	1000[a]	0.0004	1.06	5917.4	185.9	0.03	1,448,424
Furosemide	3.3 ± 2.0	80	0.01[a]	32.0	1.03	0.09	190.6	2025.8	14
Cimetidine	4.8 ± 0.1	200	6[c]	0.133	1.94	62.0	102.0	3.1	15,841
Atenolol	1.6 ± 0.02	100	26.5[a]	0.015	0.005	242.6	36,326.8	0.81	196
Ranitidine	2.2 ± 1.0	300	1000[a]	0.001	0.41	8800.8	480.6	0.02	560,303
Hydrochlorothiazide	2.0 ± 1.0	50	1[a]	0.200	0.26	11.0	747.0	17.9	360

C_{s}, solubility; D_{o}, dose number; A_n, absorption number; D_n, dissolution number; T_{abs}, absorption time; T_{diss}, dissolution time; and D_{abs}, absorbable dose.

[a]From Kasim et al.[4]

[b]From Amidon et al.[1]

[c]From Hwang et al.[28]

which can be written as ratio of residence time and absorption time[26].

$$A_n = \frac{P_{\text{eff}}}{R} \times \langle T_{\text{si}} \rangle = \frac{\langle T_{\text{si}} \rangle}{\langle T_{\text{abs}} \rangle}.$$

For calculation the R value of 1.7 cm and the predicted human P_{eff} (based on rat P_{eff}) were used.

Absorption time calculation

This parameter is proportional to P_{eff} through the following equation[26].

$$T_{\text{abs}} = \frac{R}{P_{\text{eff}}}.$$

Absorbable dose calculation

Absorbable dose is the amount of drug that can be absorbed during the period of transit time, when the solution contacting the effective intestinal surface area for absorption is saturated with the drug[26].

$$D_{\text{abs}} = P_{\text{eff}} C_{\text{s}} A < T_{\text{si}} >.$$

In this equation A is the effective intestinal surface area for absorption. If the small intestine is assumed to be a cylindrical tube with a radius of about 1.5 cm and length of 350 cm, the available surface area and volume are 3297 cm^2 and 2473 mL, respectively. In reality, the actual volume is around 600 mL and the effective intestinal surface area is then estimated to be about 800 cm^2 assuming the same ratio[27].

Results and discussion

Drugs used in this work and related data obtained from various sources[1,4,28] and also all calculated parameters are summarized in Table 1. Drugs used in this study were classified to the BCS on the basis of dose number (D_o) and rat jejunal permeability values, which are taken as indicative of fundamental properties of drug absorption, solubility, and permeability. On the basis of the relationship between human and rat intestinal permeability[3,11], rat P_{eff} values greater than 5.09×10^{-5} cm/s correspond to F_a > 85% while P_{eff} values smaller than 4.2×10^{-5} cm/s correspond to F_a values lower than 80%. Therefore, as it can be seen in Figure 1 a cutoff for highly permeable drugs, $P_{\text{eff(rat)}} = 5.09 \times 10^{-5}$ cm/s with a border line cutoff of 4.2×10^{-5} cm/s, can be set. Drugs with permeability in the range of 4.2–5.09e^{-5} cm/s were considered as borderline drugs. The intersections of dashed lines drawn at the cutoff points for permeability and dose/solubility ratio divide the plane in Figure 1 into four explicitly defined drug categories (I–IV) and a region of borderline. The biopharmaceutical properties of a drug determine the pharmacokinetic characteristics as below:

$$\textbf{\textit{Class I}}, \quad D_{\text{o}} < 0.5, \quad P_{\text{eff(rat)}} > 5.09 \times 10^{-5} \text{ cm/s}.$$

The drugs in this category are highly soluble and highly permeable and are ideal candidates for oral delivery. These drugs are characterized by the high A_n,

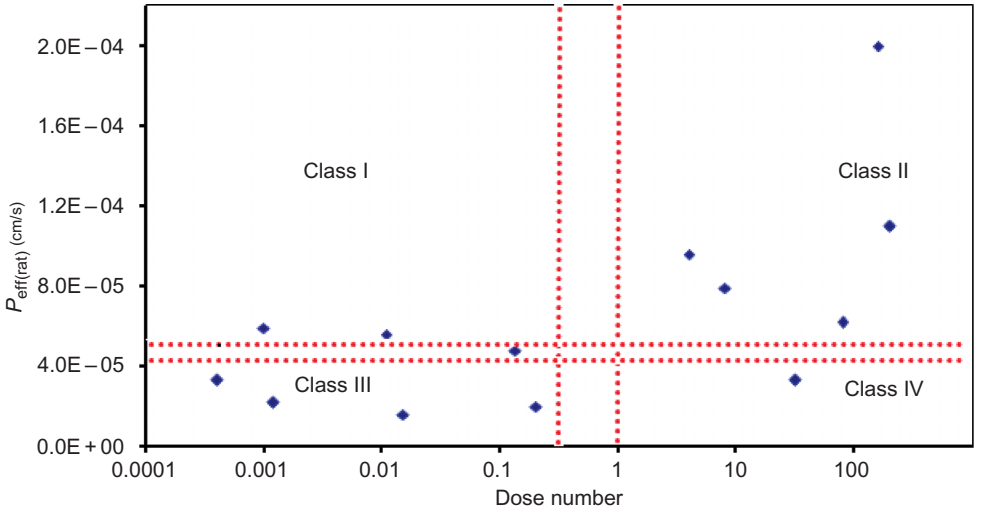

Figure 1. Plot of dose number versus rat P_{eff} values representing the four classes of tested compounds.

high D_n, and low D_o, showing that they are in solution form throughout the intestine and are available for permeation. Therefore, the rate of absorption of drugs in this class is controlled only by gastric emptying. Examples of this category include antipyrine and propranolol.

Class II, $D_o < 1$, $P_{eff(rat)} > 5.09 \times 10^{-5}$ cm/s.

Class II drugs have high lipophilicity and therefore are highly permeable across the gastrointestinal membrane, primarily by passive transport. These drugs are characterized by mean absorption time less than mean dissolution time, and thus gastric emptying and gastrointestinal transit are important determinants of drug absorption[26]. These drugs are expected to have a dissolution-limited absorption and an IVIVC is expected[2]. Low dissolution rate of these molecules limit the concentration at the site of absorption, thereby leading to less passive diffusion. Therefore, formulation plays an important role in the rate and extent of intestinal absorption of such drugs. Although there are methods to enhance the solubility of class II drugs[29,30], incorporation of polar groups into the chemical backbone, salt generation, and prodrug approaches are the primary methods for improving deliverability during lead optimization. This class includes drugs such as ketoprofen, naproxen, ibuprofen, piroxicam, and carbamazepine.

Class III, $D_o < 0.5$, $P_{eff(rat)} < 4.2 \times 10^{-5}$ cm/s.

The absorption of class III drugs is limited by their intestinal permeability and no IVIVC should be expected. These drugs are having either unfavorable physicochemical properties leading to less intrinsic permeability and/or are strong substrates to efflux transporters and/or gut wall metabolic enzymes[26]. Therefore, the rate and extent of intestinal absorption may be controlled by drug molecule properties and physiological factors rather than pharmaceutical formulation properties[7]. They must possess optimum lipophilicity in order to permeate the lipophilic epithelial cell membranes lining the gastrointestinal tract. Thus, for highly polar compounds, administration of less polar and more lipophilic prodrugs may improve absorption. Balance between the hydrophilicity and the lipophilicity should be maintained during incorporation of lipophilic groups into the structure. Atenolol, hydrochlorothiazide, and ranitidine are examples of drugs in this group.

Class IV, $D_o < 1$, $P_{eff(rat)} < 4.2 \times 10^{-5}$ cm/s.

Low and variable absorption for these drugs (e.g., furosemide in this work) is anticipated because of the combined limitation of solubility and permeability. Formulation may improve the bioavailability of these drugs. However, they are compromised by their poor intestinal membrane permeability. These drugs are more likely susceptible to *P*-gp efflux and gut metabolism, as the concentration of the drug in the enterocytes at any given time will be less to saturate the transporter[26]. Strategies to improve both solubility and permeability should be worked out for these molecules, which may not be an easy task. However, obtaining this type of quality information will certainly improve drug design and help in optimizing candidates with 'brick-like' properties.

Borderline class, $0.5 < D_o < 1$ or $4.2 \times 10^{-5} < P_{eff(rat)} < 5.09 \times 10^{-5}$ cm/s.

For drugs lying in this region, bordered by the dashed lines of the four cutoff points, the predictions become more uncertain. Cimetidine, which is supposed to be in class III, has been classified in this region. All in all, 11 of 13 test drugs (85%) are correctly classified with respect to their rat P_{eff} values; however, metoprolol, a drug with high permeability, was classified as a low permeability drug belonging to class III in the presented plot (false negative). Furthermore, there are some more fundamental parameters describing oral drug absorption. These parameters include absorption number, dissolution number, absorption time, and dissolution time[26]. There is also an extra parameter named absorbable dose that was calculated to propose the absorption limiting steps in oral absorption of tested drugs. Three dimensionless parameters (D_o, A_n, and D_n) that were shown in Table 1 can be used in qualitative classification of drugs. The four BCS classes of drugs were defined on the basis of these three parameters. For easy comparison Table 2 was set such that the dimensionless parameters for each class of drugs were compared. This classification is in accordance with quantitative classification model, which was given in the first part of current section, that is, all compounds lie in the same class as did in quantitative classification. For example,

Table 2. Qualitative classification of drugs based on dimensionless parameters.

Class	Solubility	Permeability	Dimensionless parameters
I	High	High	$A_n\uparrow D_n\uparrow D_o\downarrow$
II	Low	High	$A_n\uparrow D_n\downarrow D_o\uparrow$
III	High	Low	$A_n\downarrow D_n\uparrow D_o\downarrow$
IV	Low	Low	$A_n\downarrow D_n\downarrow D_o\uparrow$

Symbols↓ and↑ represent low and high quantity for parameters.

atenolol with a D_o = 0.015 (low), A_n = 0.005 (low), and D_n = 242 (high) is classified in class III, which is in agreement with above-mentioned quantitative biopharmaceutics classification system (QBCS). Again metoprolol with A_n of 1.06 lies in class III as it did before in quantitative model. However, this is a false-negative result, as it was known to have a high permeability belonging to class I. Another interesting aspect of using these dimensionless parameters is to determine the absorption limiting steps that were summarized as a framework in Table 3. As it was mentioned before, the mean small intestinal transit time was found to be 199 minutes with a SD of 78 minutes[27,31]. This means that as a worst case, the small intestinal transit in some individuals may be only 43 minutes (mean small intestinal transit time—2 SD). The time of 50 minutes was used as a reference time of dissolution to determine whether the dissolution is fast enough to permit complete dissolution in the small intestine[27]. The $P_{eff(rat)}$ was set at 4.2×10^{-5} cm/s, which based on our correlations corresponds to over 80% of dose absorbed. Table 3 provides distinguishing conditions under which each limiting case occurs. Considering these conditions, antipyrine and propranolol meet the criteria for no-limited absorption. All of these three drugs belong to class I. However, cimetidine, a drug which was false positive in our previous quantitative and qualitative classification, lies in no-limited class again. On the other hand based on dissolution time, permeability, and absorbable dose for furosemide, a drug of class IV, its absorption would be limited by all three parameters. Therefore, it takes place in the last class of Table 3. Furthermore, drugs with low permeability which have a high absorbable dose and low dissolution time, such as ranitidine and hydrochlorothiazide (class III), are classified in permeability-limited category. Finally, the drugs of remaining class of BCS (class II) are divided into two groups based on their relative values of dimensionless parameters. All of these drugs have high dissolution time (Table 1), but regarding the absorbable dose, their absorption could be dissolution- or solubility-limited. For instance, piroxicam and ketoprofen lie in dissolution-limited class, whereas naproxen is placed in solubility-limited category.

Conclusion

According to obtained results and proposed classification for drugs, it is concluded that drugs could be categorized correctly based on dose number and their P_{eff} values in rat model using single-pass intestinal perfusion technique. This classification enables us to remark defined characteristics for intestinal absorption of all four classes using suitable cutoff points for both dose number and rat effective intestinal permeability values. Therefore, the classification of drugs using their intestinal permeability values in rats can help pharmaceutical companies to save a significant amount in development time and to reduce costs. Moreover, it could be used as a regulatory tool to substitute in vivo BE studies by in vitro dissolution tests. However, this work relies on only 13 compounds through which their P_{eff} values in rat were measured and to confirm whether the proposed classification the larger data set is needed.

Declaration of interest: The authors report no conflicts of interest.

Table 3. Absorption limiting steps and their corresponding conditions.

Absorption limiting step	Condition	Comments	Examples
No limited	T_{diss} < 50 minutes $P_{eff(rat)} > 4.2 \times 10^{-5}$ D_{abs} >> Dose	There is no limitation in drug absorption as all three parameters are in acceptable range	Antipyrine, propranolol, cimetidine
Dissolution limited	T_{diss} > 199 minutes $P_{eff(rat)} > 4.2 \times 10^{-5}$ D_{abs} >> Dose	Although solubility itself imparts to poor dissolution, the dissolution here mainly refers to particle size. The absolute bioavailability increases with increasing dose	Ketoprofen, piroxicam
Solubility limited	T_{diss} > 199 minutes $P_{eff(rat)} > 4.2 \times 10^{-5}$ D_{abs} < Dose	Solubility-limited absorption occurs mainly when a high dose saturates part of the gut. The absolute bioavailability does not increase with increasing dose	Ibuprofen, carbamazepine, naproxen
Permeability limited	T_{diss} < 50 minutes $P_{eff(rat)} < 4.2 \times 10^{-5}$ D_{abs} >> Dose	This limiting step is considered for highly soluble drugs dosed in solutions: assume no precipitation occurs. The absolute bioavailability increases with increasing dose	Ranitidine, atenolol, metoprolol, hydrochlorothiazide
Dissolution-permeability-solubility-limited	T_{diss} > 199 minutes $P_{eff(rat)} < 4.2 \times 10^{-5}$ D_{abs} < Dose	Drug absorption is limited by all steps including solubility, permeability, and dissolution	Furosemide

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