Linear Correlation of the Fraction of Oral Dose Absorbed of 64 Drugs Between Humans and Rats

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The preliminary work was presented at the meeting of the Expert Panel on Biopharmaceutics Drug Classification System of the Food and Drug Administration on October 21, 1997 at Food and Drug Administration in Rockville, Maryland. The content of this paper was also presented under the title, "Drug absorption studies in vivo in rats for predicting fraction of drug absorbed in humans" at the AAPS Workshop on Permeability Definitions and Regulatory Standards for Bioequivalance, August 17–19, 1998, Arlington, Virginia.

KEY WORDS: oral absorption; rats; humans; inter-species.

INTRODUCTION

Good prediction of the fraction of oral dose absorbed in humans (F_h) is very important during early drug development, as well as in the proposed new biopharmaceutical drug classification (1). In recent years, emphasis has been focused on using intestinal permeability in rats (1–6), jejunal permeability in humans (1–6) and Caco-2 cell permeability (7–9) for absorption prediction and correlation. In spite of their successes, these methods generally exhibit a steep slope of correlation between F_h values of 0.1 to 0.8 and effective permeability. Such phenomena would potentially make F_h prediction less accurate, especially when a high variation in permeability is not uncommon. The permeability or absorptive clearance (3,4,11) methods have been very useful in studying mechanisms of absorption (1,2,4,7–11). The use of Caco-2 cell monolayer is especially valuable for high throughput screening of a large number of compounds, as it can quickly rank order compounds for their absorption potential.

Although rat is probably the most common animal employed in preclinical oral absorption studies, it appears that no study to quantitatively correlate the extent of absorption between human and rat for a wide variety of compounds has ever been published to date. It is of great interest to note that the mean jejunal permeability of several compounds was recently reported to be 3.6 times higher in humans than in rats (2). The rationale behind this difference and its significance in terms of fraction of oral dose absorbed between the two species has not been reported to date.

The purpose of this communication is to report our finding of great similarity in oral absorption of 64 drugs with wide physicochemical and pharmacological properties and with F values ranging from zero to unity between the two species. This finding indicates that evaluation of in vivo absorption in rats may be employed as an alternate method to predict the extent of oral absorption in humans. The present study was partly prompted by findings in our laboratory in the last fifteen years that the fraction of dose absorbed in rats (F_r) is very similar to F_h for seven drugs with absorption ranging from a few to 100% (12,13 and other uncited references).

METHODS

A total of 64 drugs were selected for comparison in this study. Initially about 70 drugs with absolute bioavailability (this may be quite different from the extent of absorption due to first pass metabolism in gut wall and liver) in humans ranging from about 5 to 100% and with a wide variety of physicochemical and pharmacological properties were randomly selected from an extensive pharmacokinetic table (14). After a literature search and evaluation on the extent of gastrointestinal absorption in humans (F_h) and in rats (F_r), some 45 drugs with absorption data were identified. The reason for not selecting the other drugs was not due to any bias but due to lack of conclusive data on the extent of absorption either in rats or humans in the reported literature for these drugs. The extent of absorption reported in the literature or estimated by us (only for a few drugs) was based on studies using radiolabeled compounds or based on standard pharmacokinetic methods. Data for additional drugs was obtained from review of other limited references. In this study, the dose fraction absorbed was found or assumed to be in the linear range even though the absorption of drugs such as verapamil or levodopa may involve carrier-mediated mechanisms. For this type of drugs the rate of absorption may be different but the extent of absorption may be same or very similar when different doses are used. It is also generally assumed that dissolution rates from the dosage form used did not significantly affect the extent of absorption. In the present study, drugs with F_h or F_r values equal to or greater than 0.9 were reported to be rapidly absorbed.

It is to be noted that drugs known to be unstable in the gastrointestinal lumen such as erythromycin and penicillins were excluded from the study; the drugs used in the present study are generally known to be stable in the gut. Data on absorption of drugs, which are known to be affected by particle size (such as nitrofurantoin) and by polymorphism (such as chloramphenicol palmitate) were also excluded. The extent absorption of chlorothiazide (13) and gabapentin are known to be dose dependent. In the present study the highest fraction of dose absorbed presumably in the linear range in both species was selected for correlation.

RESULTS AND DISCUSSION

The absorption data of 64 drugs in humans and rats are summarized in Table I, and the correlation between F_h and F_r is shown in Fig. 1. In humans, 40 drugs had F_h greater than 0.9 or near unity, 10 drugs had values between 0.5 and 0.9, eight drugs between 0.2 and 0.5, and six drugs below 0.2. When all 64 drugs were considered for regression analysis, there was a highly significant ($p < 0.0001$) correlation between humans and rats ($r^2 = 0.971$) with a slope near unity, indicating that the fraction of the dose absorbed between humans and rats is

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Table I. Summary of Data[a] on the Fraction of Oral Dose Absorbed in Humans and Rats for 64 Drugs

Drug	Mol. Wt.[b]	Property[c]	Absorption (% dose)[d] Humans	Rats
Antipyrine	188	N	100	100
Caffiene	194	N	100	100
Camazepam	372	B	99	97
Carfecillin	477	A	100(92–108)	95
Cefadroxil	381	A	100	95 ± 4.6
Cimetidine	252	B	98 ± 2	100
Cisapride	466	B	100	100
Clofibrate	243	N	96 ± 1	100
Diclofenac	318	A	100	100
Ethinyl Estradiol	296	N	100	100
Felodepine	384	B	100	100
Fenclofenac	297	A	100	100
Granisetron	312	B	100	100
Imipramine	296	B	100	100
Ketoprofen	254	A	100	100
Ketorolac	255	A	100	87
Levodopa	197	Z	100	97.2 ± 4.9
Acetaminophen	151	A	100	98 ± 15
Morphine	285	B	100	100
Nizatidine	332	B	100	100
Oxatomide	427	B	99 ± 1	100
Phenglutamide	288	B	100	100
Progesterone	315	N	100	100
Propranolol	259	B	100	99
Saccharin	183	A	97 ± 1	100
Salicylic Acid	138	A	100	100–101
Sormodren	366	B	100	100
Sultopride	354	B	100	100
Tolmesoxide	214	B	100	100
Verapamil	454	B	100	100
Viloxazine	237	B	100	100
Ximoprofen	—	—	100	100
Clonidine	230	B	95	100
Codeine	397	B	95	100
Flumazenil	303	B	95	100
Isradepine	371	B	92(90–95)	100
Theophyline	180	B	96 ± 8	97 ± 3.8
Hydrocortisone	362	N	95	95(82–108)
Naproxen	230	A	94	92
Venlafaxine	277	B	92 ± 8.1	97 ± 2.7
Gabapentin	171	Z	80 ≥75	79 ≥71.2 ± 3
Captopril	217	Z		
Ranitidine	314	B	61 ± 13	63 ± 2.9
Hydrochlorthiazide	297	A	71 ± 15	65
Sumatriptan	295	B	62	50 ± 4
Enalapril	376	B	59–73	34 ± 3.7
Furosemide	331	Z	61 ± 17	60 ± 6
Terbutaline	225	Z	60	60
Chlorothiazide	296	A	56	60
Atenolol	266	B	50	48–50
Azithromycin	749	B	37	45
Benazepril	425	B	37	50 ± 17
Cyclosporine[e]	1202	B	35(8–60)	39.5 ± 10.8
Lovastatin	405	N	30	29 ± 9.5
Bromocriptine	654	B	28	32–40
Nadolol	309	B	20.4 ± 2.1	18 ± 2.8
Bretylium	414	B	23 ± 9	20
Acyclovir	225	Z	20(15–30)	21
Adefovir	—	Z	12	8
PEG 900	900	N	10	2.5–4.1
Enalaprilate	384	Z	9	11
Amphotericin B	924	Z	5	5
Iothalamate	613	B	1.9 ± 0.5	4
PEG 4000	4000	N	0	1.7

[a] The sources of references will be available upon request.
[b] Molecular weight.
[c] N: Neutral compound, A: weak acid, B: weak base and Z: zwiterionic compound.
[d] Parenthesis indicating range or standard deviation, human absorption data of many compounds obtained from the 1997 edition of Physicians Desk Reference.
[e] A more recent study from Dr. Leslie Benet's lab has shown more complete absorption in humans than earlier reports, due to first pass gut metabolism; this confirms well with preliminary studies in rats conducted in our laboratory (to be published).

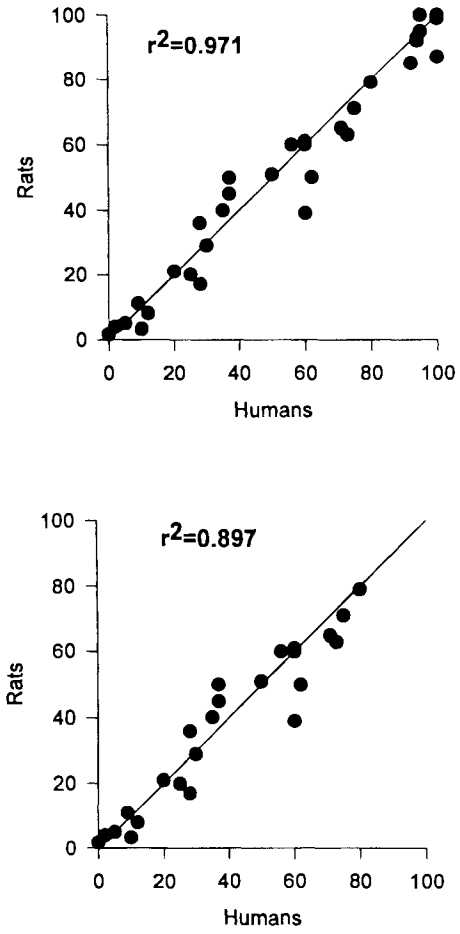

Fig. 1. Correlation of percent of oral dose absorbed between humans and rats for all 64 drugs (top), with regression equation of $F_{(r)} = 0.990F_{(h)} + 0.164$, and for the 24 drugs with less than 90% absorption (bottom), with regression equation of $F_{(r)} = 0.912F_{(h)} + 2.225$. Most data for complete absorption are not shown in the figure.

very similar for these drugs. It is of interest to note that all the 40 drugs found to be virtually completely absorbed ($F_h \geq 0.9$) in humans (4), are also completely absorbed in rats except for ketorolac with an F_r of 0.87. It is to be noted that this might be attributed to experimental methodology since the drug was also found to be completely absorbed in mice and dogs. When 24 drugs with $F_h < 0.9$ were separately analyzed, a similar high correlation ($r^2 = 0.897$; $p < 0.0001$) and a slope of 0.981 were found. The remarkable similarity in absorption between humans and rats for each of the remaining 24 drugs with F_h below 0.9 is somewhat unexpected.

The 64 drugs reported in this communication vary widely in their physicochemical properties. The molecular weights of these drugs range from 151.2 for acetaminophen to 4000 for polyethylene glycol 4000. Chemically, 9 drugs are neutral, 12 weak acids, 33 weak bases and 9 zwiterionic compounds. The lipophilicity of the compounds expressed as the logarithm of partition coefficient between octanolol and water also vary tremendously, for e.g. −5.1 for PEG4000 and 3.98 for progesterone.

Although similarity in oral absorption among human and laboratory animals has often been reported, this may represent the first study to show near identical oral absorption between humans and rats of a very large number of drugs with markedly different physicochemical and pharmacological properties and with a wide range of intestinal permeability. The present findings may be particularly significant in view of the facts that the human and rat studies were often reported from different laboratories, and the dosage forms used between the two species were often different. The present study indicates that in the dose-independent absorption range, rat may be generally used as a reliable animal model to predict the extent of GI absorption in humans following oral administration of a drug in solution or in rapidly released dosage form. The accuracy for the 40 virtually completely absorbed drugs in the present study is near 100%.

In most of the previous correlation studies, only one to four drugs with F_h values between 0.1 and 0.8 were employed. Since the slope of correlation in this F_h range is quite steep, it appears that studies with additional drugs may be needed for more rigorous validation of the method. In contrast, a total of 20 drugs in this F_h range have been employed in the present study. The same slope of correlation in the entire absorption range may be considered to be a major advantage of using F_r to predict F_h. It is of interest to note that permeability through Caco-2 cell monolayer was not found to be predictive of in vivo absorption for four different peptide-like drugs in humans and that no correlation was found between F_h and lipophilicity of nine passively absorbed drugs (15).

The nearly identical fraction of drugs absorbed between humans and rats appears intriguing in light of reported marked differences in intestinal permeability (2), in thickness of unstirred water layer in the lumen, which is about 25 μm for humans (16) and 106 μm for rats (16), in the small intestinal length, which is about 5 meters in humans (4) but is only 0.8 meters in rats (4), and in the small intestinal radius, which is about 10 times larger in humans compared to rats (2). In addition, the transit time of the small intestine is known to be different in rats and humans. It would be of interest to see whether similar findings can also be found in other rodents like mouse.

A recent report on bioavailabilities of 16 PEG compounds with molecular weights ranging from 280 to 950 has been shown to be similar between rats and humans. Since, these oligomers are minimally metabolized and the first-pass metabolism after oral dosing is expected to be insignificant or minor, their bioavailability profiles (ranging from 0.14 to 0.79 in humans and from 0.21 to 0.56 in rats), should reflect the absorption profiles (17). Their bioavailability data (obtained from their Figure 4) is shown in Fig. 2, The correlation coefficient is 0.975 with $p < 0.0001$, indicating that the rat may serve as a good animal model to predict oral absorption in humans for these neutral, hydrophilic compounds. In contrast, dog appears to be less predictive for these compounds as shown in Fig. 2.

An obvious disadvantage of using rat to predict oral absorption in human is the difficulty of using this method to screen hundreds or thousands of potential drug candidates in the early phase of drug discovery and development compared to the in vitro methods such as using Caco-2 cells and intestinal tissues. The greatest advantage of this method is that it is an in vivo method that can give us an idea of the absorption from the entire GI tract. In this regard, it is also quite different from commonly used jejunal perfusion methods in animals and

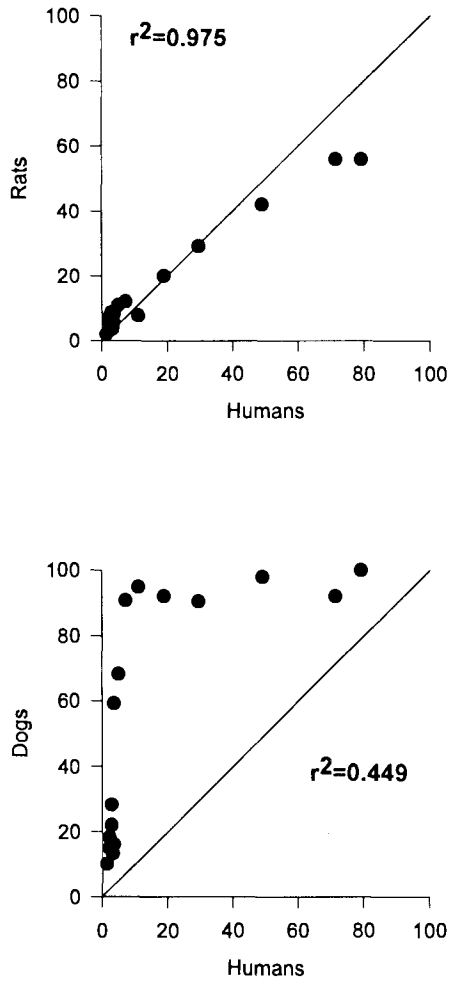

Fig. 2. Correlation of oral bioavailability of 16 PEG oligomers between humans and rats (top), with regression equation of $F_{(r)} = 0.697F_{(h)} + 4.861$, and between humans and dogs (bottom), with regression equation of $F_{(r)} = 0.973F_{(h)} + 38.994$.

humans, as these methods may not succeed if drug absorption varies greatly with different sites of absorption or with the presence or absence of bile salts. Also, marked difference in absorption from the same site of different species of animals has been reported in literature (18).

Although the mass balance method (5) has been commonly employed to study the extent of GI absorption, a relatively simple method may be employed for routine screening or evaluation in rats (12). The method involves analysis of drug in the entire GI lumen at about 6 to 8 hours after intravenous or oral dosing, provided the drug is reasonably stable in the lumen. This method is based on the assumption that absorption of most of the drug should normally occur within 6 to 8 hours as the transit time of the small intestine, the major site for absorption of most drugs, is only about 2.5 hours (13). The information obtained from intravenous dosing is to correct for GI exsorption or excretion of drug after absorption. After that correction, the fraction of dose not recovered in the lumen is approximated to equal the fraction of dose absorbed. This method has been successfully used to study absorption of furosemide (12), amphotericin B and cyclosporine in our laboratory. To avoid the potential complication of biliary excretion, absorption study may be conducted in bile-duct cannulated rats. To study the initial rate of absorption i.e., the relative magnitude of permeability or absorptive clearance for drugs that are predominantly absorbed in the small intestine, rats may be sacrificed for analysis of drug content in the GI tract one to three hours after dosing (12). For drugs absorbed significantly or predominantly in the large intestine, rat may be sacrificed 24 hours after dosing.

CONCLUSIONS

The present study indicates that evaluation of in vivo absorption in rats may be used as an alternative method to satisfactorily predict the extent of GI absorption in humans following oral administration of drugs in a solution or rapidly released dosage form. The apparent linearity in absorption between humans and rats is validated with 64 compounds having vastly different physiochemical and permeability (absorption from zero to unity) properties. A simple method to estimate the F value in rats for preliminary evaluation is described.

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