JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 803–828

Received October 13, 2006
Accepted February 13, 2007
DOI 10.1211/jpp.59.6.0007
ISSN 0022-3573

Prediction of human pharmacokinetics—evaluation of methods for prediction of hepatic metabolic clearance

Urban Fagerholm

Abstract

Methods for prediction of hepatic clearance (CL_H) in man have been evaluated. A physiologically-based in-vitro to in-vivo (PB-IVIV) method with human unbound fraction in blood ($f_{u,bl}$) and hepatocyte intrinsic clearance (CL_{int})-data has a good rationale and appears to give the best predictions (maximum ~2-fold errors; < 25% errors for half of CL-predictions; appropriate ranking). Inclusion of an empirical scaling factor is, however, needed, and reasons include the use of cryopreserved hepatocytes with low activity, and inappropriate CL_{int}- and $f_{u,bl}$-estimation methods. Thus, an improvement of this methodology is possible and required. Neglect of $f_{u,bl}$ or incorporation of incubation binding does not seem appropriate. When microsome CL_{int}-data are used with this approach, the CL_H is underpredicted by 5- to 9-fold on average, and a 106-fold underprediction (attrition potential) has been observed. The poor performance could probably be related to permeation, binding and low metabolic activity. Inclusion of scaling factors and neglect of $f_{u,bl}$ for basic and neutral compounds improve microsome predictions. The performance is, however, still not satisfactory. Allometry incorrectly assumes that the determinants for CL_H relate to body weight and overpredicts human liver blood flow rate. Consequently, allometric methods have poor predictability. Simple allometry has an average overprediction potential, > 2-fold errors for ~1/3 of predictions, and 140-fold underprediction to 5800-fold overprediction (potential safety risk) range. In-silico methodologies are available, but these need further development. Acceptable prediction errors for compounds with low and high CL_H should be ~50 and ~10%, respectively. In conclusion, it is recommended that PB-IVIV with human hepatocyte CL_{int} and $f_{u,bl}$ is applied and improved, limits for acceptable errors are decreased, and that animal CL_H-studies and allometry are avoided.

Clinical Pharmacology, AstraZeneca R&D Södertälje, S-151 85 Södertälje, Sweden

Urban Fagerholm

Correspondence: U. Fagerholm, Clinical Pharmacology, AstraZeneca R&D Södertälje, S-151 85 Södertälje, Sweden. E-mail: urban.fagerholm@astrazeneca.com

Acknowledgements: My colleagues at AstraZeneca R&D Södertälje for inspiration, and Marcus Björnsson for reviewing the manuscript and for valuable suggestions.

Note: This paper includes personal opinions of the author, which do not necessarily represent the views or policies of AstraZeneca.

Introduction

Background

Hepatic clearance (CL_H) is an important determinant of the pharmacokinetics. Good predictions of CL_H in man are essential for selecting suitable candidate drugs, and for assuring safety in early clinical studies. Several methods for prediction of CL_H (and CL) in man exist, including simple interspecies extrapolation, allometric scaling with and without correction for brain weight and maximum life-span potential (MLP), allometric scaling with correction for species differences in in-vitro intrinsic CL (CL_{int}) and/or unbound fraction (f_u), in-vitro to in-vivo correlation, physiologically-based in-vitro to in-vivo (PB-IVIV) prediction, and in-silico methods. These methodologies all have their weaknesses and strengths, both regarding rationales, assumptions and performances. There is no global consensus as to which models are most appropriate and whether these are assumed sufficiently good, and comparisons between methods are few.

Objectives

The objectives were to: evaluate the different approaches for prediction of CL_H in man (including re-analysis and re-evaluation of data, results and conclusions); find/define the most appropriate approaches; evaluate if the performances are sufficiently good for obtaining accurate stop/go-decisions during candidate drug selection and safe and effective

dosing in early clinical studies; and investigate if further method development is required.

Simple interspecies extrapolation

Available data show that, in general, there are poor correlations between species, and animals (rat, dog and monkey) have a higher CL for xenobiotics than man.

Sawada et al (1985) correlated rat and human metabolic CL and unbound CL_{int} ($CL_{u,\ int}$) for nine weak acidic and six weak basic drugs, and found a good correlation between rat and human $CL_{u,\ int}$, and that the rat cleared the drugs 9- to 14-times more rapidly than man. The relationships between the species were described by the following equations: Human metabolic $CL = 0.10 \times$ rat metabolic $CL^{0.92}$ ($r^2 = 0.74$; $P < 0.001$) and human metabolic $CL_{u,\ int} = 0.13 \times$ rat metabolic $CL_{u,\ int}{}^{0.95}$ ($r^2 = 0.91$; $P < 0.001$).

Chiou et al (1998) found a significant correlation ($r^2 = 0.66$; $P < 0.0001$) of plasma CL (mL min^{-1} kg^{-1}) between man and rats for 52 extensively metabolized compounds. The ratios between rat and human plasma CL averaged 13 (s.d. ± 22) and ranged between 0.7 and 123. A better correlation between rats and man was observed for unbound plasma CL (CL_u) for a limited number of these substances ($r^2 = 0.94$; $n = 15$). For one-third of the compounds, rat CL (mL min^{-1} kg^{-1}) was > 10-times higher than in man, and for two compounds (4%) only, the human CL was lower than in the rat. Ward & Smith (2004) found no apparent or only a weak correlation between human and rat CL (usually plasma CL). Almost half of the 103 compounds in their study were in different CL categories (low, moderate or high) in man and rats. These findings are supported by data obtained by Clarke & Jeffrey (2001), who found no correlation between human and rat liver microsome in-vitro CL_{int} values for 1127 compounds. CL ($n = 28$) and non-renal CL ($n = 13$) data in man and monkeys (rhesus and cynomolgus macaques) collected by Chiou & Buehler (2002) showed correlations between the two species ($r^2 = 0.82$ for both total and non-renal CL), and that the ratio between monkey and human CL ranged between 0.2 and 20 (for both CL and non-renal CL). Ward & Smith (2004) found a weak correlation between CL in man and monkeys. For one-third of the 103 compounds, CL in man and monkeys belonged to different CL categories. Similar CL differences have been found between man and dog (predominantly beagles). Overall, the monkey tended to be slightly more similar to man than rat or dog. For approximately half of the 103 substances in the Ward & Smith (2004) study, CL in dogs belonged to a different CL class than in man. The data collected by those authors demonstrated that 80–90% of compounds that were predicted (from animal data) to have low CL in man experimentally demonstrated low CL. Correct CL classification for drugs with moderate and high CL was lower, approximately 20–30% and 30–50%, respectively. Approximately 50–60% of compounds with high CL in man had low or intermediate CL in animals, and 5–10% of compounds that had low CL in man had intermediate or high CL in animals. For substances with CL corresponding to complete hepatic extraction in rats, dogs and monkeys, hepatic extraction ratios (E_H) in man were ≥ 0.10–0.20.

Allometric scaling

History

Mammals are quite similar with regard to various basic physical parameters and physiological processes (McMahon & Bonner 1983; Peters 1983; Schmidt-Nielsen 1984; Ritschel et al 1992; Davies & Morris 1993). The relationship between skeletal size and body weight (BW) was discussed by Galileo (Boxenbaum 1982) as early as 1637, and Rubner (1983) explored and found a relationship between basal metabolic rate (heat production and oxygen utilization) and body weight of dogs. Adolph (1949) was first to recognize relationships between 34 basic morphological, physiological and biochemical parameters and body weight of mammals. Günter (1975) found an empirical relationship of hepatic enzyme activity across species, and Krasovskij (1976) observed a high correlation of hepatic enzyme activity with body weight for a number of enzymes across 12 species, including man. Walker (1978) investigated hepatic microsomal drug-metabolizing monooxygenase activity in various species, and found a clear relationship between enzyme activity and body size. However, in that study man appeared to be deviant, with almost one magnitude lower activity than predicted. It has been observed that the liver weight, enzyme content and blood flow rate, metabolic rate (oxygen consumption), and many other anatomical and physiological variables are related by a general allometric equation:

$$Y = a \times BW^b \quad (1)$$

where Y, BW, a and b are the function to be measured (for example CL_H), species body weight, coefficient, and exponent, respectively (Dedrick et al 1970; Dedrick 1973; Kleiber 1975; Davidson et al 1986; Mordenti 1986; Ings 1990; Travis et al 1990; Campbell 1994; Lin 1995; West et al 1997; Lindstedt & Schaeffer 2002). The b-values for liver weight, blood flow rate (Q_H), liver enzyme content, metabolic rate, basal and liver oxygen consumption have been reported to be 0.87–0.92, 0.79–0.86, 0.73, 0.75, 0.73–0.76 and 0.77, respectively (Kleiber 1932, 1961, 1975; Benedict 1938; Brody 1945; Günter 1975; Weiss et al 1977; Mordenti 1986; Campbell 1994; Lindstedt & Schaeffer 2002). Although there is an allometric relationship for Q_H, the allometric principle overestimates the human Q_H by approximately two-thirds (Boxenbaum 1980). This is one of the factors that could explain the poor performance of allometric scaling of CL (see below). Other explanations are lack of apparent correlations between in-vitro CL_{int} and f_u vs body weight, brain weight and MLP (Caldwell 1981; Sawada et al 1984, 1985; Davidson et al 1986; Bogaards et al 2000; Fagerholm, unpublished data). The variance of the b-value for basal metabolic rate was estimated to be at least 20% (Kleiber 1932; Benedict 1938; Brody 1945; Davidson et al 1986). Hayssen & Lacy (1985) analysed basal metabolic rates in almost 300 mammalian species, and found an average b-value of 0.7, and noted that approximately 20% of the species fell more than 50% above or below the line. Adolph (1949) found that the correlation coefficient (r) among functions was high, often above 0.9, implying that the parameters correlate with body weight to the extent of approximately 80%. The b-values obtained in his study

ranged between 0.28 and 1.31, with values of 0.67 to 0.75 as most prevalent.

Lester & Keokosky (1967) and Videla et al (1975) found that the species metabolic rate of ethanol was proportional to $BW^{0.75}$. The simple and empirical allometric method has since then been applied and widely used for interspecies scaling of CL_H, CL, renal CL (CL_R), volume of distribution (V_D) and half-life (t½) of drugs (Mellet 1969; Klotz et al 1976; Weiss et al 1977; Boxenbaum 1980, 1982; Boxenbaum & Ronfeld 1983). According to this principle, and assuming a b-value of 0.75, the CL and CL_H per kg body weight in mice, rats and dogs should be 8-, 4- and 1.6-times higher than in man, respectively. When applying this method it is assumed that not only Q_H, but also the in-vitro $CL_{int} \times f_u$ and P_eS (permeability–surface area product) have a b-value of 0.75 or are related to body weight (if accepting that b-values for in-vivo CL_H or CL could differ from 0.75). According to in-vitro CL_{int} and f_u-data obtained in several species (considerable species differences and lack of correlation with body weight and MLP; see above), the allometric principle must be considered inappropriate for CL_H and CL. Dedrick (1973) emphasized early on that drug metabolism, in general, probably does not bear a relationship to body size. The allometric drug scaling approach has then been criticized by, for example, Yates & Kugler (1986) and Bonate & Howard (2000), and support is given by the data and evaluations presented in this report and in studies by Ward & Smith (2004) and Nagilla & Ward (2004). Bonate & Howard (2000) pointed out that there has been a large publication bias for prospective allometric scaling studies that have succeeded, that it has been more or less impossible to find publications in which this methodology has failed, and that it is not possible to determine *a priori* for which drugs allometric scaling will fail. They found that the average prediction results for CL appeared to be good, with a b-value close to 0.75, but the errors showed a large range and the CL in man for some drugs were very poorly predicted. Note: a 2-fold error (within 50% underprediction and 100% overprediction) is often used as a measure for a successful prediction (Houston & Carlile 1997). An accurate prediction is defined as a 1.0-fold error.

Allometric scaling without correction for interspecies CL_{int} and f_u differences

In reports where allometric scaling has been applied for prediction of human CL_H and CL it is clear that this methodology is associated with large errors and high incidence of b-values markedly different from 0.75, and the predictions seem to be less accurate than for other methods. Maximum observed underprediction and overprediction errors for CL or CL_H are about 140- and 5800-fold, respectively, and approximately 1/3 to 1/2 difficult to obtain a specific value since some studies do not present the actual values and some substances have been used in more than one study) of all prediction errors are more than 2-fold (Klotz et al 1976; Swabb & Bonner 1983; Bonati et al 1984; Chung et al 1985; Mordenti 1985; Owens et al 1987; Bachmann 1989; Hayton 1989; Lapka et al 1989; Paxton et al 1990; Mordenti et al 1991, 1996; Puigdemont et al 1991; Riond & Riviere 1990; Ritschel et al 1992; Hinderling et al 1993; Gascón et al 1994; Jezequel 1994; Cruze et al 1995; Lavé et al 1995a, 1996c, 1997a, 1999a, b; Grene-Lerouge et al 1996; Mahmood & Balian 1996a, b, c, 1999; Obach et al 1997; Sanwald-Ducray & Dow 1997; van Hoogdalem et al 1997; West et al 1997; Chiou et al 1998; Mahmood 1998, 1999, 2001, 2002b, 2004; Björkman & Redke 2000; Feng et al 2000; Hu & Hayton 2001; Liu & Chen 2001; Påhlman et al 2001; Ward et al 2002; Luttringer et al 2003; Mahmood et al 2003; Wajima et al 2002; Yamasaki et al 2004; Caldwell 2004; Lepist & Jusko 2004; Nagilla & Ward 2004; Ward & Smith 2004; Bae et al 2005; Ito & Houston 2005; Shim et al 2005; Tang & Mayersohn 2005, 2006a). The compound with the largest overprediction error (5800-fold), the protein kinase inhibitor UCN-01, was predicted to have a CL 35-fold greater than the Q_H (Tang & Mayersohn 2006a). The observed CL was less than a percent of the Q_H. This is consistent with an even greater (infinite) underprediction of the systemic exposure. Potential implications of such a misprediction are that the compound is not selected as a candidate drug or, if the compound is chosen, there is considerable overdosing in the first study in man. The b-values for CL or CL_H in these reports (several hundred compounds) ranged between −1.2 and 2.2, of which approximately 10% was greater than 1. This means that for 10% of the substances man had higher CL or CL_H values per kg body weight than smaller animals. For approximately one-quarter of all the compounds the b value was greater than 0.85 (as for Q_H), indicating that the E_H is greater in man than in animals for many compounds. The values of CL_H and CL in man are sometimes predicted to exceed the Q_H, which is impossible for CL_H. The average b-value for 54 compounds in a report by Chiou et al (1998) was 0.66 ± 0.19. For 20 substances with high plasma CL (≥ 8 mL min^{-1} kg^{-1}, which is ≥70% of plasma Q_H in man), the b ranged between 0.52 and 0.95, which is more narrow and closer to the value for Q_H than for 34 substances with low and intermediate plasma CL, 0.14–1.06. Hu & Hayton (2001) found that there was no correlation between body weight and CL for 24 (21%) of 115 substances (with CL data from at least three species), and that the average ± s.d. and range for b for the other 91 compounds were 0.74 ± 0.16 and 0.29 to 1.2, respectively. Most of these 91 compounds (81%) had values that did not differ significantly from 0.67 or 0.75. Ward & Smith (2004) compared the predictive success of simple allometric scaling and interspecies extrapolation for 103 substances, and found neither approach very accurate. The study demonstrated that CL-data from several animal species were less successful at predicting CL in man than from an individual species, and that addition of CL-data from a second non-rodent did not (adding dog data when rat and monkey data were already available) or only slightly (adding monkey data when rat and dog data were already available) improved the predictions. Liu & Chen (2001) developed a new allometric equation based on body surface area and compared the predictability of human CL for 30 drugs vs the traditional body-weight-based allometric method. The methods appeared to be comparable, with two-thirds of predictions within 2-fold of observed values and maximum errors of approximately 10. After this analysis had been done another global examination of allometry was published (Tang & Mayersohn 2006b). The results were in good agreement with those obtained in this study. Tang & Mayersohn (2006b) found that the mean, median and maximum prediction errors

for CL (n = 114) were 3.4-fold (overprediction; > 50-fold when including data for UCN-01), 1.5-fold (overprediction) and 54-fold (overprediction), respectively. Two-thirds of predictions were overestimations. Approximately 20% of compounds with low or moderate CL were predicted to have a CL above an acceptable limit (> 80% E_H). Thus, there is a potential risk that many suitable candidate drugs will be incorrectly rejected when applying this methodology. Human data for some incorrectly rejected compounds are probably missing, and therefore, the actual number could be even higher than 20%. Sixty-eight, 32, 18, 10 and 6% of predictions had prediction errors greater than 1.3-fold (a reasonable limit, at least for low CL_H-compounds), 2-, 3-, 5- and 10-fold, respectively. As an approximation, this method has a 95% confidence interval of one and a half magnitude (3-fold underprediction to 16-fold overprediction). The rank order was poor. When using the average CL_H of the drugs in this data set (500 mL min^{-1}) as a default CL_H-value the performance was only slightly poorer than allometry. With this approach, 42% of predictions were within 2-fold and predictions for 38% of the compounds were better than those obtained with allometry.

Another way of scaling CL_H and CL allometrically is to include the concept of physiological time (Carrel 1931; Brody 1937; Fischer 1966). The physiological time is defined as a species dependent unit of chronological time needed to complete a species independent physiological event (Boxenbaum 1982). Humans live seven times longer than dogs, and therefore, one year in a dogs life and seven years in a humans' life are considered equivalent physiological times. Dedrick et al (1970) was first to apply this concept in pharmacokinetics. Attempts have been made to relate $CL_{u,\,int}$, CL or CL_H to MLP or brain weight, but overall, the use of these variables does not seem to improve the predictability (Hussain et al 1993a; Mahmood & Balian 1996c; Lavé et al 1997a, 1999a; Mahmood 1998, 1999, 2004; Ward et al 1999, 2002; Björkman & Redke 2000; Liu & Chen 2001; Nagilla & Ward 2004). Nagilla & Ward (2004) evaluated the performances of various allometric scaling methods, including the standard, MLP and brain weight methods, for 103 compounds (the same compounds as in Ward & Smith (2004)). They found that with these approaches, 18 to 53% of human CL predictions were within 2-fold, and that none of the added correction factors resulted in substantially improved results. Such a finding is anticipated based on the apparent lack of correlation between CL_{int} and f_u vs body weight, brain weight or MLP (see above). Lavé & Coassolo (1998) have commented on this approach. Mahmood (2005) disagreed with the conclusion by Nagilla & Ward (2004) that empirical correction factors do not improve the allometric predictions. This is despite the facts that the simple allometric approach had a higher percentage of predictions within 2-fold than the MLP and brain weight based methods, and that the maximum observed overpredictions and underpredictions for standard, MLP and brain weight methods were approximately 70- and 140-fold, 25- and 250-fold, and 30- and 220-fold, respectively.

The allometric technique has also been applied to scale the oral CL (CL/F; where F is the oral bioavailability) and CL_u/F for compounds that cannot or have not been given intravenously (Mahmood 1997; Feng et al 2000; Mahmood 2002a; Wajima et al 2003; Tang & Mayersohn 2006b). This approach is questionable because: the allometric principle does generally not work well for CL and CL_H (see above); there is virtually no relationship of F between species (Sietsema 1989; Chiou & Barve 1998; Chiou & Beuhler 2002; Poggesi 2004; Fagerholm & Björnsson 2005); the fraction absorbed (f_a) from the gastrointestinal (GI) tract does not appear to follow an allometric relationship (Lennernäs 1997; Chiou & Barve 1998; Chiou et al 2000; Chiou & Beuhler 2002; Davis & Riley 2004); species differences of gut wall enzyme levels and patterns, and extraction exist (Cao et al 2006); and both CL and F (which is determined by CL_H) cannot show linear allometric relationships. It is not surprising that Wajima et al (2003) found that more than half of the allometrically scaled CL/F values for 87 drugs had an error of more than 2-fold, and the predictive errors ranged from 74-fold underprediction to 500-fold overprediction. Tang & Mayersohn (2006b) demonstrated that the average and median prediction error (overpredictions) with this approach were 4.2- and 1.5-fold, respectively (n = 24). In a draft document, the US Food and Drug Administration (which has a mission to protect the public health by assuring drug safety) proposes that this principle is useful for estimating the maximum starting dose and ensure safety in first time studies in man (FDA; http://www.fda.gov/cder/guidance/3814dft.pdf). Their proposed default safety factor (human dose equivalent for no observed adverse effect levels in the most sensitive animal species divided by a factor of 10) is considerably smaller than the observed errors for CL/F. The rationale, assumptions and performance strongly indicate that such an approach should not be used.

Allometric scaling with correction for interspecies CL_{int} and f_u differences

To improve the predictability of the allometric scaling principle, corrections for species differences in in-vitro CL_{int} and/or plasma f_u have been included (Boxenbaum 1980; Bonati et al 1984; Sawada et al 1985; Chiou & Hsu 1988; Paxton et al 1990; Chiou & Choi 1995; Lavé et al 1995b,1996a, b, 1997a, 1999a; Obach et al 1997; Sanwald-Ducray & Dow 1997; van Hoogdalem et al 1997; Mahmood 1998; Richter et al 1998; Ward et al 1999; Feng et al 2000; Terelius et al 2001; Zuegge et al 2001; Luttringer et al 2003; Yamasaki et al 2004; Tang & Mayersohn 2005). Chiou & Hsu (1988) found good predictability of CL_u (from rat to man) and poor predictability of CL for 15 extensively metabolized compounds. The average b-value for CL_u-data in that study was 0.66 ± 0.09. Boxenbaum & Fertig (1984) scaled the $CL_{u,\,int}$ for antipyrine in 15 mammalian species, including man, allometrically, and found –67 to +58% differences between observed and scaled values in these species. van Hoogdalem et al (1997) also found an improvement when allometric scaling of a compound was done using CL_u-data. The human CL was overpredicted 17- and 2-fold when CL and CL_u data from animals were used, respectively. Boxenbaum (1980) and Bonati (1984) scaled the $CL_{u,\,int}$ for antipyrine and caffeine, respectively, but found poor prediction of the human values. Obach et al (1997) observed no apparent difference vs traditional allometric scaling when interspecies f_u differences were included in their allometric scaling of CL for 17 substances. Similar results

were demonstrated for allometrically scaled CL_u and CL for 10 substances by Lavé et al (1997a, 1999a). Ward et al (1999) also corrected for f_u differences and failed to produce adequate scaling of the CL of their substance. In a study by Feng et al (2000), b-values for CL_u (n=26) and CL (n=34) were reported to be 0.44–1.47 and 0.39–2.15, respectively. For compounds with significant variation in f_u between species, an improvement of predictions was found when using CL_u. Likewise, predictions were not improved for substances with similar f_u across species. Despite an overall improvement, 22% (8 of 37 compounds) still had a ratio between predicted and observed CL_u between 3.6 and 39. Tang & Mayersohn (2005) corrected human and rat CL-values for species differences in plasma f_u and found that the fraction of compounds (n=61) with more than 2-fold prediction error decreased from 54% for CL to 23% for the f_u-corrected CL. The maximum under- and overpredictions decreased drastically. They also found that incorporation of plasma protein binding improved the prediction of the CL of UCN-01 (Tang & Mayersohn 2006a). Despite an improvement, the overprediction error was still 1750-fold.

Lavé et al (1999a) presented cases where the CL_{int} correction allometric scaling approach had been used, and found that 82% of CL predictions (n=11) had errors less than 2-fold. This was better than the traditional allometric approach (n=38), which demonstrated that about half of the predictions had an error greater than 2-fold. Lavé et al (1997a) demonstrated that inclusion of in-vitro hepatocyte CL_{int} data led to more accurate predictions of CL. In that study, six of 10 compounds scaled using the traditional allometric approach had errors greater than 2-fold, while all compounds had errors within 2-fold when correction for CL_{int} differences was made. The CL_u of one substance in that set was, however, underpredicted by 60-fold when using this CL_{int} correction approach. Underprediction of CL_u of similar size was shown by Lavé et al (1999a). Mahmood (1998) found that inclusion of in-vitro hepatocyte CL_{int} (data taken from Lavé et al (1997a)) improved the allometric predictions. For the 10 substances presented in his report, traditional and in-vitro CL_{int}-corrected allometric scaling showed 40 and 80% predictions within 2-fold error, and 12 and 2-fold maximum errors, respectively. Zuegge et al (2001) used allometric scaling with correction for in-vitro hepatocyte CL_{int} to predict the CL_H for 22 compounds, and found 68% of predictions within 2-fold error and a maximum error of 10-fold. Yamasaki et al (2004) demonstrated that in-vitro hepatocyte CL_{int}-corrected allometric scaling, but not allometric scaling (with and without brain weight and MLP correction), predicted the human in-vivo CL of a low CL compound well. Terelius et al (2001) used allometry for both observed and scaled (from hepatocyte data) in-vivo data, and concluded that pooling of data from different sources (in-vivo and in-vivo) was required to accurately assess the uncertainty in the predictions.

Although corrections for differences in CL_{int} and/or f_u may improve the accuracy of predictions vs the traditional allometric scaling method, this approach has some deficiencies. A correction for such differences is already considered for substances with b-values significantly different from 0.75, and in such cases, inclusion of in-vitro CL_{int} and f_u data would imply a double-correction. Furthermore, the CL_H for highly extracted compounds is less dependent on CL_{int} and f_u (more dependent on Q_H) than for substances with low and moderate CL_H, and binding to blood cells and extrahepatic CL are generally not considered.

In-vitro to in-vivo correlation

Correlations between in-vitro and in-vivo liver metabolism data have been used for prediction of the in-vivo CL_H, but due to a neglect of some essential factors predictions are poor (Kroemer et al 1992; Hoener 1994; Houston 1994; Ubeaud et al 1995; Lavé et al 1997b; Ito et al 1998; Iwatsubo et al 1997; Clarke & Jeffrey 2001; Lau et al 2002; Masimirembwa et al 2003; Yamasaki et al 2004).

Lavé et al (1997b) showed a log–log correlation between human hepatocyte in-vitro CL_{int} and human in-vivo E_H for 19 compounds. The prediction errors for low E_H compounds were quite large (up to 10-fold), and there was an overlap of in-vitro CL_{int}-values for compounds belonging to different E_H classes (low, moderate and high E_H). Lau et al (2002) compared human hepatocyte in-vitro CL_{int} and in-vivo CL for 26 compounds and found a linear correlation ($r^2=0.87$). (Note: a linear relationship between CL_{int} and CL_H is, however, not expected.) In all cases except one (where the ratio was 2.6 units), the ratios between in-vivo CL and in-vitro CL_{int} for highly extracted compounds were close to 1 unit. A larger variability was demonstrated for low extraction compounds, where the ratios ranged between 0.015 and ≥4 units. In that study, similar comparisons were made for rats, dogs and monkeys, and quite large errors were found in those species (especially for low CL compounds). Yamasaki et al (2004) found that in-vitro hepatocyte CL_{int} data could not predict the human in-vivo blood CL of their dopamine D_2-antagonist well. However, they found that the in-vitro to in-vivo correlation approach was more accurate than simple and modified allometric methods. Clarke & Jeffrey (2001) compared rat in-vitro (microsomes) and in-vivo CL for 1163 compounds, and found a scattered picture without apparent correlation between the two variables. A similar result was found for in-vitro half-life (t½) obtained using liver 9000 *g* supernatant (S9) vs in-vivo blood CL for 48 substances in rats (Masimirembwa et al 2003). The observed inaccuracies for all methods presented in this section could, at least partly, be explained by the fact that binding parameters and Q_H were not considered.

Physiologically-based in-vitro to in-vivo prediction

The commonly used PB-IVIV prediction approach requires a liver extraction model that accurately describes the mixing and convection of the blood in the liver (which is unknown), in-vitro CL_{int} and f_u-data, scaling factors for conversion of in-vitro to in-vivo CL_{int}, and Q_H-data.

Liver extraction models

Several theoretical concepts have been developed for description, understanding and prediction of hepatic drug metabolism.

Ideally, a hepatic extraction/metabolism model should: accurately present the physiological processes that regulate hepatic extraction/metabolism; be broadly applicable to a variety of drugs; and contain a minimum number of parameters (Saville et al 1992a). To have all these features in a single model is virtually impossible. To be physiologically accurate, a model must be mathematically complex and such models are difficult to use in a predictive fashion. These also require a large amount of data. When a mathematically simpler model is desired, it is necessary to make some approximations and to delete parameters assumed to be of less importance.

The well-stirred (or venous equilibrium) model (equation 1) (Rowland et al 1973) and the parallel-tube (or sinusoidal perfusion) model (equation 2) (Winkler et al 1974), which are mathematically simple (they contain three parameters: Q_H, CL_{int} and f_u in blood ($f_{u,bl}$))) and easily applied, have been commonly used and often compared in terms of their predictions of hepatic elimination (Pang & Rowland 1977; Jansen 1981; Ahmad et al 1983). These two models view the liver as two separate compartments, the sinusoid and the hepatocyte. The physiology of the liver suggests that the extent of mixing in the organ could fall between these extremes (well-stirred and parallel-tube models) (Saville et al 1992a).

The well-stirred model:

$$CL_H = (Q_H \times CL_{int} \times f_{u,bl})/(Q_H + CL_{int} \times f_{u,bl}) \quad (2)$$

The parallel-tube model:

$$CL_H = Q_H \times (1 - e^{-CLint \times fu,bl/QH}) \quad (3)$$

The well-stirred model views the liver as a well-stirred compartment, and therefore, the drug concentration in the sinusoids is assumed to equal that in the hepatic vein (Pang & Rowland 1977). The parallel-tube model assumes that the liver is composed of a series of identical and parallel tubes, along which drug concentration decreases progressively in the direction of the hepatic blood flow (Pang & Rowland 1977). It also assumes first-order liver uptake and that the concentration governing the uptake rate is the logarithmic mean sinusoidal concentration (Pang & Rowland 1977). The well-stirred and parallel-tube models are functionally equivalent if the E_H is low (< 0.5), but differ at higher E_H (Pang & Rowland 1977; Pond & Tozer 1984; Houston & Carlile 1997; Iwatsubo et al 1996, 1997). For compounds with intermediate and high E_H, the well-stirred model gives a lower CL_H than the parallel-tube model (Pang & Rowland 1977; Jansen 1981; Pond & Tozer 1984; Iwatsubo et al 1996, 1997; Houston & Carlile 1997). As an example, for compounds with E_H-values of 0.10, 0.30, 0.50, 0.70 and 0.90 with the well-stirred model, the corresponding parallel-tube values are 0.10, 0.35, 0.63, 0.90 and 1.00, respectively. The maximal model-dependent over/under-prediction potential for CL_H is therefore ~30%. The choice of model has an even greater impact on the oral F for high CL_H compounds (Pond & Tozer 1984; Iwatsubo et al 1996).

In addition to Q_H, CL_{int} and $f_{u,bl}$, the CL_H is also a function of vascular dispersion caused by the heterogeneity in sinusoidal blood flows and interconnection between sinusoids, association rate constant (k_{ass}) and dissociation rate constant (k_{diss}) (for binding to components of blood and liver), diffusion, hepatocyte permeability (P_e) (active and passive) and transport from liver tissue back to the blood circulation. The well-stirred and parallel-tube models are not suitable for description and studies of drugs for which hepatic metabolism is influenced or limited by these factors.

The dispersion model:

$$CL_H = Q_H \times (1 - (4a / ((1+a)^2 \times e^{[(a-1)/2D_N]} - (1-a)^2 \times e^{[-(a+1)/2D_N]}))) \quad (3)$$

where $a = (1 + 4R_N \times D_N)^{1/2}$ and $R_N = f_{u,bl} \times CL_{int} / Q_H$

The dispersion model (equation 3), another commonly used model, is mathematically more complex than the well-stirred and parallel-tube models, and describes the hepatic metabolic process in terms of convective flow, axial dispersion (mixing of blood), and disappearance of drugs by elimination, assuming a linear diffusional membrane transport (Roberts & Rowland 1985, 1986a). Like all hepatic elimination models this model does not faithfully describe the precise hepatic vasculature and physiology (Saville et al 1992a; Anissimov & Roberts 2002). Nevertheless, it has been successfully used to describe the steady-state linear elimination of several compounds in the perfused rat liver (Roberts & Rowland 1986a, b, c, d). The model has been proven also to be superior to the well-stirred and parallel-tube models for highly extracted drugs in the rat (Roberts & Rowland 1986c; Iwatsubo et al 1996; MacGregor et al 2001). The model is characterized by two dimensionless parameters, the dispersion number (D_N) and the efficiency number (R_N). These parameters are generally obtained in liver perfusion studies in animals. A limitation has been limited understanding and mathematical description of the D_N (Anissimov et al 1999). When dispersion is very high ($D_N \rightarrow \infty$) or very low ($D_N \rightarrow 0$), the profile approaches that expected for the well-stirred and parallel-tube models, respectively (Roberts & Rowland 1986a). Rat liver perfusion data suggest that the dispersion appears to be closer to the parallel-tube model (D_N often 0.2–0.5 (Roberts & Rowland 1986b)) than to the well-stirred model. When the dispersion model is used for estimation and prediction of CL_H, regardless of species (including man), the D_N-value is often set to 0.17 (as suggested for rats by St-Pierre and Weiss (St-Pierre et al 1992)) (Iwatsubo et al 1997; Ito et al 1998; Naritomi et al 2001, 2003; Shibata et al 2002; Ito & Houston 2004). D_N-values in animal species other than rats are rare, and estimates for man have not been possible to find. According to Saville et al (1992a), larger D_N-values than 0.17 must be used for species larger than rats (the human liver has a greater sinusoid network than the rat liver). Thus, when setting the D_N to 0.17 for prediction of CL_H in man, the dispersion model might overpredict the CL_H, especially for compounds with intermediate and high hepatic CL_H.

More complex models, which include more determinants for hepatic extraction, have been developed (Saville et al 1992a). It has not been possible to find a model that takes all the descriptors of hepatic drug extraction into account. These models are most commonly used for analysis of animal liver perfusion data (Chiou 1984; Weisiger 1985; Lee & Chiou 1989a, b; Roberts et al 1990; Schwab et al 1990; Weisiger et al 1991; Goresky et al 1992, 2000; Saville et al 1992b;

Evans et al 1993; Hussein et al 1993b; Piekoszewski et al 1993; Proost et al 1993; Xu et al 1994; Pang et al 1995; Mellick & Roberts 1996, 1999; Chow et al 1997; Kwon & Morris 1997a, b; Ott et al 1997; Ott & Weisiger 1997; Hung et al 1998, 2001, 2004; Anissimov et al 1999; Haddad & Funk 2001; Anissimov & Roberts 2002; Niro et al 2003; Sahin & Rowland 2004; Siebert et al 2004; Liu & Pang 2005). These creative and complex models are of great value for the understanding of the mechanisms determining the hepatic drug metabolism in animals, but have rarely been used for prediction of CL_H. It appears that the models have been mainly created and used in academia, and not in the pharmaceutical industry. Haddad & Funk (2001) developed a model for prediction of hepatic metabolism in the rat. In their model, the liver was divided into seven heterogenous segments, where each segment was subdivided into sinusoidal space, space of Disse and the liver cells. CL_{int}-data were obtained using microsomes, and initially hepatocyte P_e was not included in the model. To improve the predictability of one of the three test compounds, hepatocyte uptake and CL_{int}-data were incorporated in the model at a later stage. Development of more complete hepatic extraction models (than the well-stirred, parallel-tube and dispersion models) for man is limited by the difficulty (of impossibility) to obtain data on the convective and dispersive forces and P_eS (or absorption CL) in the healthy human liver. Based on the dispersion model, Niro et al (2003) developed a model for prediction of human CL_H that takes the radial diffusion in the space of Disse, P_e of the sinusoidal endothelium, cell density, cross-sectional sinusoidal and space of Disse S, and total and unbound drug in the blood circulation and space of Disse into account. The D_N, hepatic transit time (TT_H) and space of Disse thickness were set to 0.10, 3–5 s and 9–12 μm, respectively. The D_N value was lower than commonly used for predictions (0.17) and that suggested for man (see above), the TT_H, which was taken from rat studies, was considerably lower than anticipated in man (20 s; Fagerholm, unpublished data), and the space of Disse thickness was considerably thicker than that found by others, 2 μm (rat data) (Blouin 1977; Forker & Luxon 1982). Furthermore, the P_e of the sinusoidal epithelium was calculated as the ratio of the molecular weight-based diffusion coefficients (D) and space of Disse thickness. Thus, it appears that it was the P_e of the space of Disse, rather than that of the endothelium, that was calculated. In their study, bound and unbound drug were given the same D. Since the CL_{int}-estimates were taken from hepatocyte experiments (the membrane P_e included as a determinant) it must have been assumed that the P_e of the endothelium plus space of Disse was lower than that of the hepatocyte membrane. Only highly permeable substances were included in the study, and the hepatocyte membrane P_e does not appear to limit the metabolism of such substances (rat data) (Miyauchi et al 1993; Fagerholm unpublished data).

Hepatic blood flow rate

Commonly used estimates of Q_H are 1.35 and 1.5 L min^{-1} (Rowland & Tozer 1995; Riley et al 2005). The Q_H in males and females of normal weight (70–75 and 60–65 kg) is ~1.5–1.6 and ~1.3–1.4 L min^{-1} respectively (Leggett & Williams 1995; Lindstedt & Schaeffer 2007).

Estimation of the in-vitro CL_{int}

The metabolic capacity is often quantified as the CL_{int}, which is a concept introduced by Wilkinson and Shand (Wilkinson & Shand 1975; Rowland 1984). Hepatic in-vitro CL_{int}-data are normally obtained using fresh or cryopreserved isolated hepatocytes, cultured (including monolayer cultures on collagen and sandwich-cultures) hepatocytes, liver slices or subcellular fractions, such as microsomes and S9. Buffer, buffer containing serum albumin, and serum are commonly used incubation media in such experiments. Each of these systems has their own advantages and weaknesses. Due to several differences, these systems produce different in-vitro CL_{int}- and predicted in-vivo CL_H-data.

Subcellular fractions, including microsomes

In-vitro CL_{int}-estimates obtained in experiments using subcellular fractions (such as microsomes) could be misleading. This is because some drug metabolizing enzymes normally present in hepatocytes are lacking or are underexpressed, several drug-binding cell compartments are lacking, or a cell membrane to permeate through is missing (Wortelboer et al 1990; Donato et al 1993; Kern et al 1997; Lavé et al 1999a; Obach 2001; De Graaf et al 2002; Lau et al 2002; Gómez-Lechón et al 2003; Masimirembwa et al 2003; Plant 2004). Microsomes only contain enzymes localized in the endoplasmic reticulum, which for drug metabolizing enzymes equates to cytochrome P450s (CYP450s), flavin monooxygenases and UDP-glucuronosyltransferases (UGTs) (Plant 2004). Metabolites produced via other enzymes (many phase II enzymes) will not be found in microsome and S9 metabolism studies (De Graaf et al 2002; Lau et al 2002). Therefore, the use of subcellular fractions is generally limited to compounds metabolized mainly by phase I enzymes. The absence of a cell membrane indicates that microsomes are not suitable for compounds with low and moderate P_e. For example, the cell membrane P_e is rate-limiting for the metabolic rate of enalaprilat in the perfused rat liver (Schwab et al 1990). Binding to various cell components influences the K_m, metabolic rate and CL_{int}, and the lack of many of these components may therefore give erroneous CL_{int}-estimates. Drug–drug interaction data obtained with microsomes could also be misleading. For example, the influence of a compound with low P_e and extensive binding to various cell components on the metabolism of another compound could be overestimated.

Liver slices

In-vitro CL_{int}-data can also be obtained using slices of the liver. A drawback of this method is limited diffusion, and underestimation of CL_{int} (Ekins et al 1995; Houston & Carlile 1997; Haenen et al 2002).

Isolated hepatocytes

Hepatocytes are intact cells with an outer cell membrane through which drugs must pass to reach the intracellular metabolizing sites, and with a complete set of phase I and II drug metabolizing enzymes and co-factors (Fabre et al 1990; Cross & Bayliss 2000; Brandon et al 2003; Donato & Castell 2003; Gómez-Lechón et al 2003, 2004; Blanchard et al 2004). These are advantages that make them closer to the in-vivo

situation than subcellular fractions. Disadvantages with isolated hepatocytes include a decline of viability, decline of expression and activity of many specific proteins (including the CYPs and transporters), loss of normal cell attachment and polarity, and large surface area (S) compared with the in-vivo situation (Schwenk 1980; Groothuis & Meijer 1996; LeCluyse et al 1996; Liu et al 1999; Reinoso et al 2001; Gómez-Lechón et al 2003; Blanchard et al 2004; Chandra & Brouwer 2004). After 4 h in culture in a study by Gómez-Lechón et al (2003), hepatocytes had lost 70–90% of their CYP mRNA levels. This decline preceded decreased CYP content and activity. This team also showed that 75–100% of CYP2C9, CYP2E1 and CYP3A4 activity was remaining at 24 h after culturing (Gómez-Lechón et al 2003). Skett (1994) found that isolated hepatocytes maintained viability for approximately 4 h. The in-vitro conditions might lead to decreased active drug transport (Schwarz et al 1979; Schwenk 1980; Blom et al 1982; Olinga et al 1998; Houle et al 2003). Other drawbacks with isolated human hepatocytes are limited availability, technical demand, phenotype instability and high interindividual variability (Gómez-Lechón et al 2003).

Cryopreservation is a method available for long-term storage of freshly isolated hepatocytes. Some critical parameters for successful cryopreservation are source of the liver, choice of a suitable cryoprotectant, composition of cryopreservation medium, cellular density, and cooling and thawing rates (Gómez-Lechón et al 2003). It has been shown that serum in the freezing medium does not influence the results (Li et al 1999), but that tight packing (cell density 5×10^6 cells mL^{-1}) during cryopreservation reduces the viability and metabolic activity (De Loecker et al 1998). The metabolic activity of cryopreserved and fresh hepatocytes has been compared, and the activity for phase I metabolic enzymes appears to be similar or lower in cryopreserved hepatocytes, whereas some phase II metabolic enzymes in the cryopreserved medium demonstrate lower activity for some substrates (Li et al 1999; Steinberg et al 1999; Hengstler et al 2000; Railland et al 2000; Hewitt et al 2001; Lau et al 2002; Griffin & Houston 2004; McGinnity et al 2004). Houle et al (2003) showed that activity might decrease 60% after cryopreservation, McGinnity et al (2004) found that cryopreserved human hepatocytes had on average 6% lower in-vitro CL_{int} than fresh cells, and Naritomi et al (2003) demonstrated that for two of seven substances, the in-vitro CL_{int} was more than 2-times lower in cryopreserved than fresh rat hepatocytes. Griffin & Houston (2004) demonstrated that, for four of 14 metabolic pathways, in-vitro CL_{int}-values obtained using cryopreserved rat hepatocytes were between 1/25 to 1/5 of corresponding values obtained with fresh hepatocytes. Lau et al (2002) found that cryopreserved hepatocytes from dog and monkey, but not from rats, gave similar average in-vitro CL_{int}-values as fresh cells. Their cryopreserved rat hepatocytes demonstrated on average 4.7-fold lower in-vitro CL_{int} than fresh cells, and the maximum ratio between in-vitro CL_{int} data obtained with fresh and cryopreserved rat hepatocytes was 15. Blanchard et al (2005) found that the recovery of viable human hepatocytes from three donors after 3-h preservation at 4°C or after cryopreservation were 53–90, and 0–50%, respectively. Sohlenius-Sternbeck & Schmidt (2005) demonstrated that rat hepatocytes had lost 90% of intracellular glutathione levels and more than 90% of glutathione activity after cryopreservation. Cryopreserved cells from man and rats showed similar levels and activity. The relationship between viability and activity has not been well studied. In the study by Blanchard et al (2005) there was no apparent difference in CL_{int}-values for three substances between fresh and cryopreserved human hepatocytes (except for a batch without viable cryopreserved cells), which indicated that a relationship between viability and activity might not necessarily be linear. Similar indications were demonstrated by Gómez-Lechón et al (2003) (see above).

Cultured hepatocytes

In contrast to isolated hepatocytes, cultured hepatocytes develop intact canalicular networks, retain protein expression and function, re-establish polarized excretory function, and have an apical S that is similar to that of the liver in-vivo (Liu et al 1999; Chandra & Brouwer 2004). Maintenance of function and differentiation for up to 4–6 weeks has been found (Kono et al 1997; Weiss et al 2002). A proposed drawback with this method is that drug diffusion in the collagen layers may affect the CL_{int} (Treijtel et al 2004). The metabolizing capacity of cultured hepatocytes may also decrease, but less than for isolated cells (Griffin & Houston 2005). A human hepatome HepaRG cell line is also available for studies of hepatic disposition (Le Vee et al 2006). Griffin & Houston (2005) estimated and compared CL_{int}-values for seven compounds using freshly isolated and monolayer cultured rat hepatocytes. They found that only two compounds had comparable (within 2-fold) CL_{int} in both systems, and that the monolayer culture gave higher CL_{int} for a slowly metabolized compound (warfarin) and lower (approximately one magnitude) CL_{int} for rapidly metabolized substances. According to the protocol, the medium for isolated suspended hepatocytes, but not for cultured cells, contained small amounts of bovine serum albumin (0.03%). Since warfarin is highly bound to albumin, the binding might be a reason for the lower CL_{int} of this compound found with isolated hepatocytes. A proposed explanation for the lower CL_{int} for rapidly metabolized substances in monolayer culture was an initial 50% loss of enzyme activity (relative to suspension) during the 2-h attachment period before the experiment (Griffin & Houston 2005). Those authors speculated that the low CL_{int}-values for those substances might have been uptake rate limited. At least four of those highly extracted compounds are known to have high P_e, and therefore, it does not seem likely that differences in CL_{int} between the systems had been due to differences in P_eS. The exposed S of isolated hepatocytes is 3- to 7-fold greater than that of cultured hepatocytes (Weibel et al 1969). Despite this S difference there were no apparent differences in CL_{int}-estimates obtained with fresh and sandwich-cultured cells from dogs and monkeys (Lau et al 2002). A possible explanation could be that the sinusoidal membrane has a relatively high P_e. It has been demonstrated that the canalicular membrane of hepatocytes contains more cholesterol, which makes membranes more rigid and less permeable (Meier et al 1984). Another explanation could be that the data set did not contain compounds with permeation-rate limited metabolism.

Incubation media

Buffer, buffer containing serum albumin, and serum are commonly used as incubation media in in-vitro metabolism experiments. Serum is more costly and somewhat more difficult to work with, and the disappearance of substrate is sometimes less than in serum-free media. Advantages with this approach are that the dissociation step (from drug-transporting proteins) is included as a determinant for CL_{int}, separate f_u-measurements are not required, and drug adsorption to apparatus could be reduced. The use of serum in microsome and S9 experiments must, however, be considered unphysiological.

Methods for estimation of the in-vitro CL_{int}

The in-vitro CL_{int} is commonly estimated from metabolite formation V_{max} and K_m values (metabolite formation method; MFM), or from the depletion of substrate (substrate depletion method; SDM). A drawback with the MFM is that the CL_{int} will be underestimated if not all the metabolic processes have been found and quantified. In contrast to the SDM, the MFM requires that experiments are performed at several substrate concentrations. For these reasons, the SDM is more commonly used. When the SDM is used, the CL_{int} is normally calculated using the t½ (or elimination rate constant, k_{elim}) or the ratio between concentrations at the end and start of the experiment, a standardized volume based on the volume of the medium, and the cell density (see for example, Bachmann et al (2003), Shibata et al (2000, 2002), Niro et al (2003) and Floby et al (2004)). Under the assumption that the kinetics are linear, the k_{elim} ($k_{elim} = CL_{int} \times V_D$) of a single hepatocyte is similar to that of a whole liver. The concentration-ratio between terminal and initial concentrations during an in-vitro experiment depends on both the CL_{int} and the V_D (both binding and unbinding) of the system. A compound with negligible hepatocyte binding and high CL_{int} and a substance with extensive hepatocyte binding and low CL_{int} could therefore demonstrate similar remaining concentrations in the medium at a certain time point. The V_D of the system is determined by the volumes of the medium and cells (only a small fraction; see below), and the binding to the cells. As a result of binding to hepatocytes, the actual V_D will generally be larger than the physical volume. An increase in cell density will therefore (generally) not produce a proportional increase of the V_D, as expected for CL_{int} (under the assumption that CL_{int} of one cell is independent on the CL_{int} of another cell). Thus, the k_{elim} is cell density dependent. The use of a standardized volume (such as that of the incubation medium) of the medium and k_{elim} will generate underestimated CL_{int}-values, especially when the binding to hepatocytes is extensive. For example, at a cellular density of 2×10^6 cells mL^{-1}, the CL_{int} is underpredicted by 10, 30 and 80% when the partitioning to hepatocytes is 15, 50 and 500 higher than to the medium, respectively. Underestimation of the incubation V_D could therefore be a reason for underpredictions of CL_H. Weaknesses with the SDM are uncertainty in the CL_{int}-estimates for substrates with low CL_{int} (Tucker et al 2001; Obach & Reed-Hagen 2002; van Eijkeren 2002; Treijtel et al 2004), uncertainty whether to use the terminal t½ (equilibrium phase) or the total disappearance of substrate (includes the cell uptake and binding processes) for estimation of the CL_{int}, and substrate adhesion to surfaces. For slowly metabolized compounds with low partitioning coefficient, the difference in incubation concentrations at the start and end of the experiment is small. In an in-vitro study with fresh rat hepatocytes (cell density 2×10^6 cells mL^{-1}), approximately 5 ± 0.5, 10 ± 1 and $20 \pm 1\%$ ($n = 3$) of the antipyrine content in the incubation media (William's E medium or serum) had disappeared (due to hepatocyte disposition) at 0.5, 1 and 2 h, respectively (Shibata et al 2000). The CL_{int} and plasma f_u of this compound in the rat are 7 mL min^{-1} kg^{-1} and ~1, respectively. The CL_{int} corresponds to an E_H of approximately 0.1. In a study with cryopreserved human hepatocytes, Shibata et al (2002) found less solute disappearance than with fresh rat cells. The low metabolic rates might have been due to reduced viability and activity of the cells. The incubation concentrations of four slowly metabolized compounds decreased by 2 ± 1 (5×10^6 cells mL^{-1}), 4 ± 2 (5×10^6 cells mL^{-1}), 4 ± 1 (2×10^6 cells mL^{-1}) and $9 \pm 3\%$ (1×10^6 cells mL^{-1}) ($n = 3$) during 2-h experiments. The predicted E_H for these substances was 0.02, 0.03, 0.07 and 0.28, respectively. The CL_{int}-data had to be multiplied by a factor of 3–4 to get accurate predictions of the in-vivo CL. Another cause of low solute disappearance is saturated metabolism due to high concentrations. A prerequisite for obtaining good data is that experiments are performed at physiologically relevant concentrations.

Based upon an average hepatocyte volume of 4000 μm^3 (or 4×10^{-9} mL) per cell (Gebhardt 1992), and a cell density of 1, 2 and 5 million cells (mL medium)$^{-1}$, hepatocytes would occupy approximately 4, 8 and 20 per mL of the volume of the incubation system, respectively. For a metabolically stable compound with a tissue:plasma or tissue:buffer partitioning coefficient of >50, >100 or >250 (depending on cell density), the hepatocytes would eventually absorb and retain more than half of the amount of substrate in the incubation medium. Due to absorption into and binding to hepatocytes, the initial t½ (absorption and distribution phase) for compounds with extensive binding is often shorter than the terminal t½, both in an in-vitro incubation experiment and in-vivo. This phase contains relevant information regarding hepatic absorption and disposition.

Correction for binding to microsomes and hepatocytes

Attempts have been made to consider the unspecific binding to microsomes when estimating the CL_{int} (Austin et al 2002; Grime & Riley 2006). Such binding is not necessarily reflective of the in-vivo situation (Obach 1999). For several reasons, compensation for this unspecific binding is questionable; unspecific binding to cell components is a determinant of the CL of hepatocytes; the f_u in a microsome incubation is not the f_u that is available for binding and metabolism (in contrast to the f_u in blood, which is the f_u available for hepatic uptake and disposition); and microsomes and hepatocytes have different binding compartments. Hepatocytes have a cytosol (considerable fraction of cell volume), an outer membrane in which compounds could reside (especially basic compounds), and organelles, such as lysosymes and mitochondria, which are capable of binding lipophilic bases extensively (Siebert et al 2004). Austin et al (2005) demonstrated that the extent of binding of several compounds to microsomes differed up to a magnitude from that of hepatocytes.

As demonstrated below, it is not clear when and how corrections should be made.

Correction for unspecific binding to hepatocytes has also been made (Austin et al 2005; Grime & Riley 2006). Such an approach is also questionable. The k_{elim} in an in-vitro experiment is determined by the V_D (two dimensions; binding during the distribution phase and unbinding during the elimination phase) and CL_{int}, rather than by the f_u and CL_{int}. The k_{elim} could also be determined by the dissociation rate from binding sites. Grime & Riley (2006) demonstrated quite large differences between in-vitro and in-vivo unbound hepatocyte CL_{int} (rat and dog data). The fitted lines deviated from unity, in-vitro data were generally underpredictive (up to a magnitude or more), and two compounds with similar in-vivo unbound CL_{int} could have two magnitudes different in in-vitro unbound CL_{int}.

Scaling from in-vitro to in-vivo CL_{int}

In-vitro CL_{int}-data need to be converted to the CL_{int} of a whole liver. This is usually done by using the data for the number of hepatocytes per gram human liver (120×10^6 cells (g liver)$^{-1}$) or the CYP450 content in hepatocytes (0.14 nmol per million cells) or microsomes (0.32 nmol (mg microsomal protein)$^{-1}$), and the liver weight (1800 g in a male adult) (Iwatsubo et al 1996). A recently established value for human liver microsomal scaling is 40 mg microsomal protein (g liver)$^{-1}$ (Hakooz et al 2006).

Estimation of $f_{u,bl}$

It is quite common that the f_u in plasma ($f_{u,pl}$), and not the $f_{u,bl}$, is used in predictions of CL_H. Binding to blood cells often occurs, especially for lipophilic bases, and therefore, a neglect of this binding could cause poor predictions. Normally, the $f_{u,bl}$ is not measured directly, but estimated from equilibrium in-vitro $f_{u,pl}$ and blood concentration/plasma concentration (C_{bl}/C_{pl})-data ($f_{u,bl} = f_{u,pl}/(C_{bl}/C_{pl})$) (Wilkinson & Shand 1975; Iwatsubo et al 1997; Ito et al 1998; Masimirembwa et al 2003). The $f_{u,bl}$ can also be estimated from $1/((1-Hct)/f_{u,pl})) + (Hct/f_{RBC}))$, where Hct and f_{RBC} are the haematocrit and fraction bound to/in red blood cells (RBCs), respectively (Pang & Rowland 1977). It is assumed that the unbound concentrations in RBCs and plasma are equal. This approach must also presume that the f_u in RBCs ($f_{u,RBC}$) contributes to the $f_{u,bl}$ and is directly available for hepatic uptake and disposition, and that there is no permeation-limit across the RBC membrane. Furthermore, the fraction of cytoplasmic water (65% of the total cell volume (Simeonova et al 2002)) is not considered. The physical distribution volume of $f_{u,RBC}$ is overestimated by ~50% when the Hct is used as a measurement for this volume. The use of this approach causes ~80% overestimation of the true $f_{u,bl}$. Considering that the blood consists of 29% RBC cytoplasmic water (Hct × 65%) and 56% plasma water (1 – Hct), and that unbound concentrations in RBCs and plasma are equal, 34% of the f_u in blood resides within the RBCs. The amount of intracellular free drug is ~½ of that in plasma. The time before entering the liver for the first time may be insufficient for reaching binding equilibrium, substance might be 'stripped' from its binding sites during passage through the liver, and the rate of dissociation might be too slow to maintain equilibrium along the sinusoids. In such cases, in-vitro equilibrium estimates of $f_{u,bl}$ could be misleading and cause poor predictions.

Predictions of CL_H

Several groups have applied the PB-IVIV approach for predictions of in-vivo CL_H and CL (Rane et al 1977; Lin et al 1978; Igari et al 1984; Bäärnhielm et al 1986; Chiba et al 1990; Singh et al 1991; Ashforth et al 1995; Hayes et al 1995; Zomorodi et al 1995; Lavé et al 1996a, 1999a; Houston & Carlile 1997; Izumi et al 1997; Obach et al 1997; Iwatsubo et al 1997; Sanwald-Ducray & Dow 1997; Carlile et al 1998, 1999a; Gagner Milchert et al 1998; Ito et al 1998; Bayliss et al 1999; Matsui et al 1999; Obach 1999; Kimura et al 2000; Obach 2000; Shibata et al 2000, 2002; Andersson et al 2001, 2004; Haddad & Funk 2001; Hirota et al 2001; Zuegge et al 2001; Kumar et al 2002; Mahmood 2002b; Soars et al 2002; Luttringer et al 2003; Niro et al 2003; Davis & Riley 2004; De Kanter et al 2004; Galetin et al 2004; Ito & Houston 2004, 2005; Lam & Benet 2004; McGinnity et al 2004; Blanchard et al 2005, 2006; Riley et al 2005; Mohutsky et al 2006). Quite often microsome CL_{int}-data have been used and binding to blood cells has been neglected. Predictions obtained with hepatocyte CL_{int} and $f_{u,bl}$-data appear to be most accurate.

Data obtained using microsomes

The PB-IVIV approach with microsome in-vitro CL_{int}-data generally underpredicts the in-vivo CL_H, and is apparently no more accurate than allometry (see Table 1). Plausible reasons have been presented above. The underprediction potential is a possible cause of failures in early clinical studies (insufficient exposure). In studies where binding to plasma proteins and blood cells have been considered, the PB-IVIV method has underestimated the in-vivo CL in man by 5- to 9-fold on average (including compounds with both low and high E_H). The maximum under- and overpredictions found are 106- and 10-fold, respectively.

Obach et al (1997) predicted the human in-vivo CL of eight compounds with this approach. They assumed that hepatic phase I metabolism was the dominating elimination process. Data on binding to plasma proteins and RBCs were included or excluded, and the well-stirred and parallel-tube liver models were applied. The various approaches demonstrated on average 60% predictions within 2-fold error, and quite large maximum errors. When f_u-data were included in the equations, large underpredictions (magnitudes) were observed for all the highly protein-bound ($f_u < 0.04$) compounds. The average-fold prediction errors for SDM and MFM were 9 and 8, respectively. There were no apparent differences in accuracy compared with allometry.

Two years later, Obach (1999) used human microsome in-vitro CL_{int} (SDM) and $f_{u,bl}$-data, and the well-stirred and parallel-tube models to predict the in-vivo non-renal CL (after intravenous dosing) for 29 substances in man. The in-vivo non-renal CL was generally underestimated to a large extent. The average and maximum (under)-prediction errors were 6.5-, 6.5- and 5-fold, and 15-, 19- and 10-fold for bases,

Table 1 Performance of commonly used methods for prediction of hepatic clearance (CL_H), non-renal CL or CL of mainly metabolized compounds

Method		A/N/B	Errors						Data extracted from
			Mean[a]	Median[a]	≤ 1.1[a]	≤ 1.3[a]	≤ 1.5[a]	≤ 2[a]	
Simple allometry[b]		A/N/B	↑↑	↑	n.d.	n.d.	n.d.	~50–67%	Several references (see text)
		A/N/B	3.4 ↑	1.5 ↑	15%	32%	50%	68%	Tang & Mayersohn (2006b)
Default	CL_H set to 500 mL min^{-1}	A/N/B	8.0 ↑	2.5 ↑	6%	22%	27%	41%	Tang & Mayersohn (2006b)
PB-IVIV human microsomes	with $f_{u,bl}$	A/N/B	5–9	n.d.	n.d.	n.d.	n.d.	15–60%	Several references (see text)
		Acids	4.8 ↓	4.8 ↓	0%	0%	22%	33%	Obach (1999)
		Neutrals	6.5 ↓	4.0 ↓	12%	25%	25%	38%	-"-
		Bases	6.5 ↓	4.0 ↓	0%	0%	0%	8%	-"-
	without $f_{u,bl}$	Acids	15 ↑	2.6 ↑	11%	11%	11%	22%	-"-
		Neutrals	2.1 ↑	2.1 ↑	0%	12%	25%	38%	-"-
		Bases	1.6 ↓	1.2 ↓	33%	58%	83%	92%	-"-
	with $f_{u,bl}$ and $f_{u,inc}$	Acids	3.4 ↓	3.3 ↓	22%	22%	33%	33%	-"-
		Neutrals	3.4 ↓	2.2 ↓	12%	25%	50%	50%	-"-
		Bases	2.0 ↓	1.7 ↓	0%	8%	42%	75%	-"-
	with $f_{u,bl}$ and scaling factor	A/N/B	~2 ↑	2 ↑	~20%	~25%	~35%	~70%	Ito & Houston (2005)
PB-IVIV human hepatocytes	with $f_{u,bl}$	–	–	–	–	–	–	–	–
	with $f_{u,bl}$ and scaling factor	A/N/B	1.4 ↑	1.3 ↑	21%	64%	64%	93%	Shibata et al (2002)
	without $f_{u,bl}$	Neutrals	2.3 ↑	1.8 ↑	15%	25%	35%	60%	McGinnity et al (2004)
		Bases	1.7 ↑	1.4 ↓	21%	48%	59%	72%	-"-
	with $f_{u,bl}$ and $f_{u,inc}$	Acids	4.7 ↓	3.5 ↓	6%	12%	12%	24%	Riley et al (2005)
		Neutrals	4.7 ↓	4.5 ↓	5%	14%	29%	33%	-"-
		Bases	1.8↓	1.5 ↓	11%	33%	56%	67%	-"-
PB-IVIV rat hepatocytes[c]	with $f_{u,bl}$	A/N/B	~1.2	~1.2	~25%	~50%	~95%	100%	Shibata et al (2000)

(Continued)

Table 1 *(Continued)*

Method		A/N/B	Errors						Data extracted from
			Max underpred[a]	Max overpred[a]	Range	Ranking	Viability	n	
Simple allometry[b]		A/N/B	140	5,800	812 000	Poor	–	> 100	Several references (see text)
		A/N/B	5	54	270	Poor	–	114	Tang & Mayersohn (2006b)
Default	CL_H set to 500 mL min^{-1}	A/N/B	20	84	1680	Poor	–	102	Tang & Mayersohn (2006b)
PB-IVIV human microsomes	with $f_{u,bl}$	A/N/B	106	10	1060	Poor	–	99	Several references (see text)
		Acids	10	–	7	Poor	–	9	Obach (1999)
		Neutrals	19	–	19	Poor	–	8	-"-
		Bases	15	–	9	Poor	–	12	-"-
	without $f_{u,bl}$	Acids	1.7	59	100	Poor	–	9	-"-
		Neutrals	2.3	3.5	8	Poor	–	8	-"-
		Bases	5	1.3	6.5	Poor	–	12	-"-
	with $f_{u,bl}$ and $f_{u,inc}$	Acids	7.5	–	7.5	Poor	–	9	-"-
		Neutrals	11	1.3	14	Poor	–	8	-"-
		Bases	4.3	–	3.4	Poor	–	12	-"-
	with $f_{u,bl}$ and scaling factor	A/N/B	6	5	30	Poor	–	33	Ito & Houston (2005)
PB-IVIV human hepatocytes	with $f_{u,bl}$	–	–	–	–	–	–	–	–
	with $f_{u,bl}$ and scaling factor	A/N/B	1.7	2.3	3.9	Good	45–60%	14	Shibata et al (2002)
	without $f_{u,bl}$	Neutrals	2.0	11	22	Poor	n.d.	20	McGinnity et al (2004)
		Bases	3.4	4.2	14	Poor	n.d.	29	-"-
	with $f_{u,bl}$ and $f_{u,inc}$	Acids	18	1.9	34	Poor	n.d.	17	Riley et al (2005)
		Neutrals	19	1.5	28	Poor	n.d.	21	-"-
		Bases	4.0	1.4	5.6	Poor	n.d.	18	-"-
PB-IVIV rat hepatocytes[c]	with $f_{u,bl}$	A/N/B	< 2	1.5	< 3	Good	> 90%	18	Shibata et al (2000)

[a]Fold-error (2-fold error = +100% or −50%; an accurate prediction is defined as a 1.0-fold error). [b]Includes compounds with various elimination routes. [c]Prediction of rat CL. A/N/B, acids/neutrals/bases; $f_{u,bl}$, unbound fraction in blood. (Note: consideration of unbound fraction in plasma generally gives higher CL_H-estimates.) $f_{u,inc}$, unbound fraction in the incubation mixture; n.d., not determined; PB-IVIV, physiologically based in-vitro to in-vivo prediction. ↓ underprediction trend, ↑ overprediction trend, ↑↑ great overprediction trend.

neutrals and acids, respectively. For bases and neutrals, predictions were improved when $f_{u,bl}$-data were not considered. In contrast, predictions of acids were worse. The average and maximum prediction errors for bases, neutrals and acids when neglecting $f_{u,bl}$ were 1.6-(mainly underpredictions), 2.1- and 15-fold (mainly overpredictions), and 5-, 3.5- and 59-fold, respectively. Attempts were made to correct for both $f_{u,bl}$ and f_u in the microsome incubation mixtures ($f_{u,inc}$). The CL_H of most compounds were underpredicted with this approach. Average and maximum errors were 2–3-fold and 4–11-fold, respectively. Overall, the ranking was poor.

Ito & Houston (2005) used human microsome CL_{int}-data, $f_{u,bl}$-data and the well-stirred model to predict the human in-vivo CL_H for 33 drugs. The CL_H was underpredicted for all except one compound. The average-fold error was ~4.5, ~15% of the compounds had less than 2-fold error, and the maximal underprediction was ~30-fold. Inclusion of an empirical scaling factor (6.2) that took the underprediction into account, led to improved predictions. Good predictions were demonstrated for approximately one-third of the compounds. An error greater than 2-fold was found for ~30% of the compounds, the error range was 25-fold, and the rank order was poor. Corresponding data for simple allometry were ~45% and ~180-fold, respectively.

Iwatsubo et al (1997) and Ito et al (1998) compared human microsome in-vitro CL_{int} (MFM) and in-vivo CL_{int} (calculated using CL in-vivo and $f_{u,bl}$-data, and well-stirred, parallel-tube and dispersion equations) for 29 substances, and found a poor correlation. The in-vivo CL_{int} for substances with low CL_{int} were underpredicted (maximum 106-fold; greater than 10-fold error for 17% of the substances (mainly acids)), and more than a 3-fold error for 50% of the compounds. The maximum overprediction error was 10-fold.

Naritomi et al (2001) used the in-vitro to in-vivo pharmacokinetic prediction approach with correction of the human microsome in-vitro CL_{int} (SDM) with a scaling factor (animal in-vivo CL_{int}/animal in-vitro CL_{int}). They compared uncorrected and corrected (vs rat and dog data) human microsome in-vitro vs in-vivo CL_{int}-values for eight model compounds (with E_H 0.03–0.87 in man). The in-vivo CL_{int} was estimated using in-vivo CL (assuming negligible extrahepatic elimination), Q_H, plasma f_u,C_{bl}/C_{pl}, and the well-stirred, parallel-tube and dispersion models. Human correction factors ranged between 0.3- and 27-fold, and the ratio between animal and human scaling factors ranged between approximately 0.5 and 4. The average (median) ratio between in-vivo CL_{int} and in-vitro CL_{int} for the well-stirred model in man was 6.5 (5). No apparent differences between the liver extraction models were observed. The effect of binding to microsomes was evaluated, and it appeared that inclusion of this parameter did not improve the overall accuracy of the predictions.

Underpredictions of in-vivo CL were also demonstrated in studies where binding to blood cells was neglected. Carlile et al (1999a) underpredicted the human in-vivo CL_{int} of four acid CYP2C9 substrates by 3- to 20-fold. They calculated the in-vivo CL_{int} from oral exposure data, assumed that the first-pass and equilibrium E_H were similar, and that the in-vitro CL_{int} was estimated in the absence and presence of 2% bovine serum albumin.

Andersson et al (2004) used in-vitro microsome CL_{int}, plasma and microsome binding data, and the well-stirred model for prediction of human in-vivo CL (estimated from plasma exposure data after oral dosing) for acid CYP2C9-substrates. They found large predictive errors, overprediction when plasma f_u was not considered, and underprediction when plasma and microsome f_u were taken into account.

Mahmood (2002b) did not consider binding to plasma proteins and blood cells when predicting the in-vivo human CL_H of 16 randomly selected drugs from human liver microsome in-vitro CL_{int}-data. The CL_H was overpredicted for 70% of the compounds, more than 50% of the predictions had more than 2-fold errors, and the maximum error was more than 10-fold. The results were comparable with those of simple allometric scaling.

Data obtained using liver slices

In general, the in-vitro CL_{int}-data obtained with liver slices are lower than for hepatocytes and they underpredict the in-vivo CL_H (Worboys et al 1995, 1996a, b, 1997; Houston & Carlile 1997; Carlile et al 1999b; Andersson et al 2001; De Kanter et al 2004). One possible explanation is the limited diffusion of compounds into the slices (Ekins et al 1995; Houston & Carlile 1997; Haenen et al 2002).

Data obtained using hepatocytes

The PB-IVIV approach with hepatocyte in-vitro CL_{int}-data and $f_{u,bl}$ have generated good predictions of the CL_H in the rat. Shibata et al (2000) obtained in-vitro CL_{int}-data of 18 compounds (mainly eliminated by hepatic metabolism) using fresh rat hepatocytes (viability greater than 90%) suspended in rat serum or William's E medium. The dispersion model and SDM were used, and plasma f_u (non-serum data only) and C_{bl}/C_{pl}-data were taken into account. To be able to quantify the CL_{int} for substances with low metabolic rates, the cell density was increased by 3- or 5-fold (from 1 to 3×10^6 cells mL^{-1} with serum, and from 0.2 to 1×10^6 cells mL^{-1} with William's E medium) and the incubation time was 2 h. The % drug disappearance and cell density were used to estimate the CL_{int} ($CL_{int}=k_{elim}\times$cell density). They found a good correlation ($r^2=0.94$) between predicted in-vivo blood CL_H (serum method data) and observed in-vivo plasma CL (obtained from intravenous pharmacokinetic data), small predictive errors (according to the correlation figure in their paper errors generally appear to be less than 50% and negligible for about half of the compounds), correct classification (low, moderate and high CL), and appropriate rank order. The relationship between predicted in-vivo blood CL_H and observed in-vivo blood CL (or CL_H) data was, however, not shown. I used available C_{bl}/C_{pl} and in-vivo plasma CL data to calculate the in-vivo blood CL, and found that for many compounds, especially those with high CL, the CL was overestimated. For the three compounds with the highest CL, the blood CL was overpredicted by a factor of approximately 2. A possible reason for this could be a twofold use of C_{bl}/C_{pl}-data in the dispersion model. The C_{bl}/C_{pl} was used for estimation of the R_N, and multiplied with the estimated blood CL. After compensation for this, the correlation and relationship between predicted in-vivo blood CL_H and observed in-vivo blood CL was

similar to that demonstrated for predicted in-vivo blood CL_H vs observed in-vivo plasma CL (see above). The 20–25% overprediction found for seven of the compounds might have been due to the use of relatively high Q_H (70 vs 56 mL min^{-1} kg^{-1}; +25%). If 56 mL min^{-1} kg^{-1} had been used the CL would not have been overpredicted. Instead, the CL for some compounds would have been underpredicted. Such an underprediction is likely to occur when extra-hepatic elimination occurs. Many of the test substances had a CL exceeding the Q_H. The relationship between predicted in-vivo blood CL_H from non-serum method data and observed in-vivo blood or plasma CL data were not shown. The in-vitro vs in-vivo CL_{int}-data for the both methods were shown and compared, and it appeared that the predictability of the serum and non-serum methods was comparable for most substances. The similar results obtained with serum and serum-free media (with f_u-correction) indicated that the dissociation of these test compounds had not been limiting (permissive binding). One exception was that the CL_{int} of tolbutamide, which is a highly albumin bound low CL compound, was one magnitude lower with the serum method. This indicates that non-equilibrium might exist for its binding.

Predictions of CL_H in man with a similar approach are, however, not as good as for the rat. Possible reasons to poor predictions include: use of cryopreserved human hepatocytes with low viability and activity (probably the main reason); use of liver extraction models that do not correctly reflect the dispersion in the human liver (underprediction potential with the well-stirred model and overprediction potential with the parallel-tube and dispersion (with a D_N of 0.17) models); incorrect estimation of the in-vivo CL_H (underestimation due to a neglect of extrahepatic elimination); underestimation of CL_{int} due to the assumption that the V_D in hepatocytes is similar to their physical volume; insensitivity of in-vitro methods to accurately estimate low CL_{int}; 80% overestimation of $f_{u,bl}$ because of the assumption that the $f_{u,RBC}$ is directly available for hepatic uptake and disposition; the assumption that binding equilibrium exists for all compounds, including those with slow association and/or dissociation rates; and different TT_H of blood components and unbound molecules (the TT_H for $f_{u,bl}$ is longer than for the bound fraction, and therefore, there is an underprediction potential for high CL_{int}-compounds with high $f_{u,bl}$). It has not been possible to find a prediction study where fresh human hepatocytes and $f_{u,bl}$-data have been used.

Shibata et al (2002) predicted the human in-vivo CL_H and CL_{int} for 14 drugs (nine bases, four neutrals and one acid) from in-vitro CL_{int}-data obtained with cryopreserved human hepatocytes suspended in human serum. In consistency with their previous study with rat hepatocytes, the incubation time was prolonged (2 h), the SDM was used, the cell density for low CL_{int} compounds was increased (5×10^6 cells mL^{-1}), C_{bl}/C_{pl} was considered, and the PB-IVIV prediction approach with the dispersion model ($D_N=0.17$; value valid for rats) was used. The % drug disappearance and cell density were used to estimate the CL_{int} ($CL_{int}=k_{elim}\times$cell density). As in their previous rat study, they corrected for C_{bl}/C_{pl} twice. In contrast to the results for predictions in the rat, the predictions generally resulted in considerable underestimation. When an empirically estimated scaling factor (the estimated biological number of hepatocytes in a liver was multiplied by a factor of 3–4) was included, good predictions were obtained. The ratios between predicted CL_H (corrected) and observed CL ranged between 0.57 and 2.3. Nine and six compounds (64 and 43%) had prediction errors <1.3- and <1.2-fold, respectively. The largest errors were found for low E_H-compounds, and the acid and neutral low CL_H-compounds generally had larger errors than bases. Predicted F_H-data correlated well with observed oral F-data. For 57% (8/14) of the compounds, the predictive error was less than 1.05-fold, and for 79% (11/14) the error was ≤1.5-fold. Possible explanations for the underpredictions have been presented above. Appropriate rank order was found for 10 of the substances, and the remainder had switched places with compounds of adjacent placing and similar CL_H (between 1 and 12% CL_H-difference). The cell viability was estimated to be 45–60%, which was considerably lower than in the rat study (>90%) where a lower maximum cell density was used (3 vs 5×10^6 cells mL^{-1}). A recent study demonstrated that cryopreserved human hepatocytes consistently underpredicted the in-vivo CL_{int} of five CYP3A4-substrates, and that it might have been due to loss of metabolic activity (Hallifax et al 2005). Shibata et al (2002) assumed that hepatic metabolism was the major route of elimination and the oral absorption was complete, and that CL_H and 1-E_H were equal to CL and oral F, respectively. However, nine of 14 compounds had incomplete GI absorption (f_a-values in man 0.80–0.99) (Palm et al 1997; Kansy et al 1998; Wessel et al 1998; Balon et al 1999; Pérez et al 2004), and extrahepatic elimination has been found for at least four of the test drugs (Thummel et al 1997; Goodman Gilman 2001; von Richter et al 2001; Glaeser et al 2004). Verapamil is substantially metabolized by CYP3A4 in the human gut wall mucosa, especially during absorption (Thummel et al 1997; von Richter et al 2001; Glaeser et al 2004). At least four other compounds in the study are metabolized by CYP3A4 (Thummel et al 1997; McGinnity et al 2004), which indicates that considerable gut wall metabolism might occur for these as well. Propranolol (one of the drugs studied) has an E_H that exceeds the $f_{u,bl}$ (Shand et al 1973), and propranolol and lidocaine (another drug studied) dissociate fast from α1-acid glycoprotein and slowly from albumin (Pardridge et al 1983). Improvements of in-vivo CL_H estimations could probably have given more accurate predictions.

Niro et al (2003) used in-vitro CL_{int} and $f_{u,bl}$-data, and the well-stirred and a modified dispersion model (radial diffusion, cell density and areas incorporated) to predict the human in-vivo CL_H of six drugs (two acids, two neutrals and two bases). Drug disappearance from suspensions (SDM; CL_{int} calculated as $k_{elim}\times$volume of the cell suspension) of human cryopreserved hepatocytes (cell density 2×10^6 cells mL^{-1}) was measured up to 6 h (a comparably long time). Cell viability studies were carried out to ensure adequate viability. These viability results were, however, not shown in their paper. The predictions based on their dispersion model were more accurate than those based on the well-stirred model. This is a bit surprising, since the liver extraction models are not expected to differ much for prediction of CL_H for drugs with low E_H (Roberts & Rowland 1986a, b, c; Iwatsubo et al 1997). E_H-values for the drugs used in this study ranged between approximately 0.01 and 0.20, and within this range,

E_H-estimates from the well-stirred and parallel-tube models (the two extremes) are not expected to differ more than 1%. The well-stirred model underpredicted the CL_H of the three drugs with highest (but still low) E_H by 30–50%, and gave approximately 30% lower CL_H values than the dispersion model for four of the test compounds. For the dispersion model, the difference between predicted and observed CL_H ranged between approximately 40% underprediction and 50% overprediction, and predicted values were on average only a few per cent higher than for the actual values. The average fold-error was approximately 1.25. Inclusion of a scaling factor of approximately 1.4 for the well-stirred model would give as good predictions as the dispersion model. Low/decreased metabolic activity of the cryopreserved hepatocytes is one of the possible explanations to the underpredictions of CL_H with the well-stirred model. In case this is true, the dispersion model is overpredictive. The model assumptions and choice of a space of Disse thickness could possibly explain why the modified dispersion model gave higher CL_H-values than the well-stirred model. For example, they used a D_N that was lower than commonly used for predictions (0.10 vs 0.17; this difference is however assumed negligible), a TT_H that was considerably shorter than anticipated in man (3–5 vs 20 s), and a space of Disse thickness that was considerably thicker than found by others (9–12 vs 2 μm) (Blouin 1977; Forker & Luxon 1982). Furthermore, the P_e-estimates of drug and drug–protein complexes across the sinusoidal endothelium were both set to equal the D of the drug divided by the space of Disse thickness. A simulation of different thicknesses of the space of Disse showed that 12 μm gave approximately 40–50% greater CL_H-values than 9 μm, and interestingly this difference is similar to the difference between the two hepatic extraction models used in the study. Apparently, their dispersion model is sensitive to the choice of diffusion and S-values.

Naritomi et al (2003) applied the same approach as they used for microsome-data (see above). Four of the nine compounds (human E_H 0.03–0.76, including conjugated substances) in the paper from 2001 were included. This time, only the well-stirred and dispersion models were applied, and in-vitro studies were performed using fresh rat hepatocytes, and cryopreserved rat and human hepatocytes. It was assumed that drug binding to blood components had equilibrated momentarily upon entering into the portal blood, that no extrahepatic elimination occurred, and that no excretion of intact and metabolized drug occurred from the intestines. Ratios between observed and predicted human hepatocyte in-vitro CL_{int} ranged between 2.2- and 136-fold and 2.8- to 199-fold for the well-stirred and dispersion models, respectively. The corresponding ranges found when the scaling factor was included were 1.3- to 8.1-fold and 1.9- to 11-fold, respectively. Thus, the in-vitro CL_{int}-data always underestimated the in-vivo CL_{int}, and the maximum errors were quite large. The effect of binding to rat hepatocytes was investigated, and for some substances, the in-vitro CL_{int} corrected for hepatocyte binding was not in agreement with the in-vivo CL_{int}. For seven compounds, the in-vitro CL_{int} obtained using fresh and cryopreserved rat hepatocytes were very similar. For two substances, however, the in-vitro CL_{int} was more than 2-times lower when using cryopreserved cells.

Soars et al (2002) used the well-stirred model, and human hepatocyte (fresh and cryopreserved; 4 million cells mL^{-1}; 90-min incubation; with 2 g L^{-1} human serum albumin) and microsome (incorporating blood-to-plasma partitioning ratio, and plasma and microsome f_u-data) in-vitro CL_{int}-data (SDM) to predict to human in-vivo CL for 11 glucuronidated compounds (glucuronidation responsible for 10–90% of in-vivo CL). The concentration of human serum albumin was approximately 20-times lower than in-vivo. The blood-to-plasma partitioning ratio was assumed to be 1 for basic and neutral compounds and 1−Hct for acids. They found that the use of microsomes, but not fresh hepatocytes, led to considerable underprediction of the in-vivo CL. A significant correlation between predicted and observed human in-vivo CL_H ($r^2 = 0.79$; $P < 0.005$) was demonstrated for eight investigated substances when using fresh human hepatocytes suspended in buffer containing human serum albumin. The presented figure shows that the CL_H for two of the low E_H compounds was not well predicted (approximate 10-fold error), and that the predicted and observed CL_H for two of the substances with high E_H (which are less dependent on CL_{int} and binding than on Q_H) differed by approximately 30%. Viability data was not presented. The correlation between in-vitro CL_{int} data obtained using fresh and cryopreserved human hepatocytes appeared to be good ($r^2 = 0.80$; $P < 0.001$). The interindividual variability for in-vitro CL_{int} appeared to be quite large, both for fresh and cryopreserved human hepatocytes.

Zuegge et al (2001) applied the well-stirred liver model and hepatocyte in-vitro CL_{int} (SDM) data to predict the human in-vivo CL_H for 22 extensively metabolized drugs. The liver was assumed to be the main metabolizing organ, and drug binding to blood cells and plasma proteins was neglected. Sixty-four and 77% of predictions had errors less than 2- and 3-fold, respectively, and the maximum fold-error was estimated to 6.

In a study by McGinnity et al (2004), the in-vitro CL_{int} of 50 neutral and basic marketed drugs were determined in fresh human hepatocytes (SDM), and used to predict the human in-vivo CL_H. The well-stirred model was used. Binding to plasma proteins was not considered, and the distribution between blood and plasma was assumed to be unity. The linear regression of the data showed a modest correlation ($r^2 = 0.48$, $P < 0.05$). The prediction error was within 2-fold for two-thirds of the compounds, and the maximum over- and underpredictions were 11- and 3.4-fold, respectively. Approximately one-third of predictions were acceptable (<30% error for low CL_H-compounds and <10% error for high CL_H compounds). Furthermore, the mean and median errors were 95 (overprediction) and 68% (overprediction), respectively. The average overprediction errors for substances with low and high E_H were 2.2- and 1.4-fold, respectively. The prediction of CL_H of neutral compounds appeared poorer. The mean and median overprediction errors for these substances ($n = 20$) were 2.3- (2.5-fold for those with low E_H; overprediction in 75% of cases) and 1.8-fold, respectively. Fifty-nine per cent of these predictions had an error < 2-fold. With this approach, nine of 29 compounds (31%) with intermediate and high CL_H could potentially have been incorrectly stopped from further development (predicted $E_H > 80\%$). There was no apparent relationship between

enzymes responsible for metabolism and predictability, and the rank order was poor (80 and 40% differed >3 and >10 placings; 22 placings maximum difference). The overprediction potential and rather poor accuracy might have been due to the assumptions regarding drug binding. The re-evaluation demonstrates a higher average prediction error for compounds with low $f_{u,bl}$. The finding that predictions for neutral compounds were poorer is consistent with their comparably low $f_{u,bl}$. The median and average $f_{u,bl}$ for neutrals were 3- and 4-times smaller than for bases, respectively. Acids generally have lower $f_{u,bl}$, and therefore, this approach is expected to be even less suitable for this class of compound. The performances of cryopreserved human and dog hepatocytes were also investigated using 14 drugs from the set. These cells retained on average 94 and 81% of the CL_{int} in fresh cells, respectively.

In a later report, Riley et al (2005) collected human hepatocyte in-vitro CL_{int}-data for 17 acids, 18 bases and 21 neutrals from its own and other groups, and corrected for both $f_{u,bl}$ and $f_{u,inc}$ when predicting (well-stirred model) CL_H. The CL_H of most compounds were underpredicted and the ranking was comparably poor. The average and maximum prediction errors for acids, bases and neutrals were 5-, 1.8- and 5-fold, and 18-, 4- and 19-fold, respectively. Only 24, 67 and 33% of predictions in each group were <2-fold. I inserted a scaling factor (multiplied predicted in-vivo estimates by the average prediction error) to investigate improvement and found that prediction errors were still quite large (average 1.6- to 2.7-fold, maximum 2.8- to 10-fold, 47 to 89% <2-fold error). Similar results were found for compounds with low E_H.

Cryopreserved human hepatocytes in suspension were used in a study by Bachmann et al (2003) to predict the in-vivo CL_H of six substances from in-vitro CL_{int}-estimates. Experiments were 6-h long, the cell density was 1×10^6 cells mL^{-1}, the SDM was used, and the CL_{int} was calculated as $k_{elim} \times$ volume of the cell suspension. The in-vitro CL_{int} was measured either in human serum or serum-free media, and the well-stirred model was used. Blood cell binding was not taken into account, and plasma protein binding was not taken into consideration for data obtained in serum-free media. Cellular viability was measured, and the relative percentage of viable cells (vs untreated cells) was used as a correction factor to normalize measured drug concentrations. The difference between predicted and observed in-vivo CL_H when serum was used ranged between approximately 30% underprediction and 50% overprediction, and the average of differences between predicted and observed values was only a few per cent. The corresponding range for data obtained with serum-free media was 50% underprediction to 10% overprediction, and again, the average difference between predicted and observed values was small. It was concluded that predictions based on serum and serum-free media were equivalent.

Blanchard et al (2006) used cryopreserved human hepatocytes incubated in 100% serum and in serum-free medium to predict the in-vivo CL_H of six substances (basic and neutral compounds metabolized by a variety of phase I and II enzymes and with a wide range of CL_H) in man. In-vitro CL_{int}-values were derived using SDM, and the well-stirred model was used. When incubations were performed with serum it was assumed that the binding to hepatocytes was similar to that in serum, whereas plasma and hepatocyte f_u-values were considered when studies were performed in the absence of serum. Binding to blood cells was neglected or C_{bl}/C_{pl} was set to unity. Average and maximum prediction errors and percentage with <2-fold error with serum were 1.7- (mainly underpredictions) and 2.1-fold and 67%, respectively. Corresponding values for serum-free medium were 9- (mainly underpredictions) and 28-fold and 17%, respectively.

Zuegge et al (2001) used hepatocyte in-vitro CL_{int} (SDM), the well-stirred model and a correction factor (relationship between in-vitro CL_{int} and in-vivo CL) to predict the human in-vivo CL_H for 22 extensively metabolized drugs. They assumed that the liver was the main metabolizing organ, and neglected drug binding to blood cells and plasma proteins. Sixty-four and 96% (all except one compound) of predictions were within 2- and 3-fold of observed values, respectively, and the maximum fold-error was 15. Overall, this approach seemed to be a bit more accurate than the other tested methods (allometric scaling with CL_{int}-correction, uncorrected PB-IVIV prediction and an artificial neural network (ANN) method).

Other methods

In-silico and statistical methods are also available for prediction of CL_H. Results demonstrate that further development is required.

Schneider et al (1999) combined in-vivo CL and hepatocyte in-vitro CL data for 22 extensively metabolized compounds, and utilized multiple linear regression (MLR) models, partial least squares regression (PLS) and ANN for evaluation of their ability to predict the in-vivo CL in man. Plasma protein binding was not considered. They found that dog hepatocyte data, and rat and dog in-vivo data were uncorrelated with human in-vivo CL, and did not contribute significantly to the prediction models. In a study by Zuegge et al (2001), the predictability of a supervised ANN method was compared with allometric scaling with correction for interspecies in-vitro CL_{int}-differences, and PB-IVIV (hepatocytes) prediction with and without a scaling factor. This method was apparently not more accurate than any other of the methods, and it showed the highest maximum error (22-fold). Gobburu & Shelver (1995) applied an ANN method to predict the pharmacokinetic properties, including CL, of a series of β-blockers, and were able to predict the pharmacokinetics for these as well. Hussain et al (1993a) developed an ANN tool for the prediction of CL for 14 compounds in humans and examined the performance vs allometry. The ANNs were apparently no better than the allometric approach. Wajima et al (2002) applied three types of regression methods to predict CL for 68 drugs in man, and compared the performance vs allometric scaling methods without correction for in-vitro CL_{int}- and f_u-differences. It was demonstrated that a MLR method using animal CL data and calculated structural parameters (molecular weight, clogP, and number of hydrogen bond acceptors) predicted the human CL better than allometric methods. With the MLR and allometric methods, approximately two-thirds of the predictions had less than 2-fold

error, and the maximum errors were 10- and 70-fold (overprediction), respectively. Wajima et al (2004) also used a method for human CL prediction where it was assumed that plasma concentration–time profiles were similar among species, and that normalized curves from various species could be superimposed. Species differences in CL_{int} and f_u were not considered. CL values for two of the four test compounds had less than 2-fold errors and the maximum error was estimated to be 2.8. Various in-silico methods are available for prediction of binding of drugs to metabolizing enzymes and metabolic activity (Ekins & Obach 2000; Ekins et al 2000, 2002, 2003; Roberts 2001; Tucker et al 2001; Boobis et al 2002; Ekins & Rose 2002; Ethell et al 2002; Ekins 2003; Lombardo et al 2003; van de Waterbeemd & Gifford 2003; Balakin et al 2004; Bugrim et al 2004; Crivori et al 2004; Hansch et al 2004; Henderson & Guzzo 2004; Penzotti et al 2004; Poggesi 2004). None of the in-silico methods seemed to produce accurate predictions of human CL_H and CL, and therefore, these need to be developed further. Ekins & Obach (2000) used pharmacophore modelling and quantitative structure activity relationship (QSAR) techniques to predict human hepatocytes in-vitro CL_{int} for 29 drugs with different physicochemical properties, and found quite large predictive errors. Ekins (2003) also used a partitioning model based on human liver microsomal CL_{int}-data of more than 800 structurally diverse molecules to predict the in-vitro CL_{int}. He found that the model was able to generate a statistically significant ranking for 41 drugs in a test set, but the 95% confidence intervals were large (±1.5 magnitude), suggesting low accuracy to predict the CL_u in man. Jolivette & Ward (2005) evaluated the potential of calculated two-dimensional molecular properties (molecular weight, molar refractivity, log octanol–water partitioning coefficient, polar surface area (PSA), number of hydrogen bond donors and acceptors, and rotatable bound count) to predict the extrapolative success or failure of rat, dog and monkey data to project the CL of 103 compounds in man. Compounds with like animal and human CL categorization (low, moderate, high) were considered qualitatively successful, and compounds with a predicted human CL with less than 2-fold error were considered quantitatively successful. In rats, dogs and monkeys, the qualitative success was 68, 66 and 73%, respectively. The corresponding quantitative success values were 44, 48.5 and 68%, respectively. Differences in some molecular properties were found between successful and failed predictions, and application of the findings could enrich the predictability of the human CL to some extent.

Conclusion

The objectives were to evaluate the different approaches for prediction of CL_H in man, find/define the most appropriate approach, evaluate if the performance was sufficiently good for obtaining accurate stop/go-decisions during candidate drug selection and safe and effective dosing in early clinical studies, and investigate if further method development was required.

Several methods for prediction of CL_H (and CL) in man are available, including simple interspecies extrapolation, allometric scaling with and without correction for brain weight and MLP, allometric scaling with correction for species differences in in-vitro CL_{int} and f_u, in-vitro to in-vivo correlation, PB-IVIV prediction with and without inclusion of a scaling factor, and in-silico methods. The performances of the most commonly used approaches are shown in Table 1.

A PB-IVIV method with $f_{u,bl}$ and hepatocyte CL_{int}-data has a good rationale and appears to give the best predictions. Available data indicate a high potential of this method to obtain accurate candidate drug stop/go-decisions, and select safe and effective clinical dosing strategies. Another advantage is that the method enables prediction of CL_H in different populations. Predictions in the rat obtained with rat $f_{u,bl}$ and fresh hepatocyte CL_{int}-data showed that prediction errors generally were <50% and negligible for about half of the compounds, and that CL classification and rank order were appropriate. PB-IVIV predictions obtained with human $f_{u,bl}$ and cryopreserved CL_{int}-data demonstrate maximum ~2-fold errors, 25% errors for half of predictions, and appropriate rank ordering. Inclusion of an empirical scaling factor (3–4) was, however, needed when CL_{int}-data obtained with cryopreserved human hepatocytes were used. Possible reasons for the underpredictions and need to include a scaling factor include: use of cryopreserved hepatocytes with low viability and metabolic activity; use of a CL_{int}-estimation method that assumes that V_D in hepatocytes equals that of the physical volume of the cells (underprediction for compounds with high partitioning coefficients); underestimation of the in-vivo $f_{u,bl}$ for compounds that bind slowly to blood components; and overestimation of the CL_H from in-vivo pharmacokinetic data (neglect of extrahepatic CL). Other reasons for poor or deviating PB-IVIV predictions are the use of: liver extraction models that do not correctly reflect the convection and mixing of blood within the liver; a method that assumes that the $f_{u,RBC}$ is directly available for hepatic uptake and disposition (80% overestimation of $f_{u,bl}$); the assumption that different blood components have similar TT_H; neglect of binding to blood cells; and insensitivity of in-vitro methods to obtain accurate CL_{int}-estimates when metabolic rate is slow ($E_H < 0.05$). Thus, an improvement of this methodology is possible and required. A neglect of $f_{u,bl}$ or incorporation of $f_{u,inc}$ does not seem to be the way to solve this. Such approaches are both physiologically incorrect and give comparably large prediction errors and poor ranking.

When microsome CL_{int}-data are used with the PB-IVIV approach, the CL_H is underpredicted by 5- to 9-fold on average, and a 106-fold underprediction has been observed. The poor performance could probably be explained by lack of complete sets of membranes to permeate through, cell components to bind to, and metabolizing and transporting enzymes, and low metabolic activity. Inclusion of scaling factors, and neglect of $f_{u,bl}$ for basic and neutral compounds improve microsome predictions to some extent. The rationales and performances are, however, not satisfying.

Allometric methods incorrectly assume that the determinants for CL_H relate to body weight, brain weight and/or MLP, and consequently, they have poor predictability. Simple allometry has an average overprediction potential, >2-fold errors for ~one-third of predictions, >1.3-fold (30%) errors for ~two-thirds of predictions, 140-fold underprediction to 5800-fold overprediction (potential safety risk) range,

and b-values ranging between −1.2 and 2.2. Improvements have been achieved when correcting for species differences in CL_{int} or f_u. These are generally modest and the approach lacks a valid rationale.

In-vitro CL_{int} to in-vivo CL correlations have not generated accurate CL predictions, and this could be explained by the fact that binding to blood components and Q_H have not been considered.

In-silico methodologies are available, but these need further development.

Insufficient exposure (and lack of sufficient and retained pharmacological activity) in early clinical studies could potentially have been due to underpredictions of CL with allometry, and cryopreserved hepatocyte, microsome and liver slice CL_{int}-based PB-IVIV. Underprediction of systemic exposure, which is common with allometry and when neglecting binding to blood components, is a potential safety and attrition risk factor.

A 2-fold prediction error could be sufficient for leading to attrition, and misinterpretation of the pharmacokinetic profile, elimination routes and interaction potentials. Allometry could give CL_H- or CL-values close to or above the Q_H (thus, no or negligible predicted oral F) despite good in-vitro stability, and thereby suggest a rejection of suitable candidate drugs. The oral F for compounds with high CL_H is very sensitive to $CL_{int} \times f_{u,bl}$ prediction errors and the choice of liver extraction model. Maximum prediction errors of ~50% at low CL_H and ~10% at high CL_H are desirable and reachable.

In conclusion, it is recommended that PB-IVIV with human hepatocyte CL_{int} and $f_{u,bl}$ is applied and improved, limits for acceptable errors are decreased, and that allometry and animal CL_H-studies are avoided. With this approach, high quality CL_H-predictions, and improved candidate drug selection, lead optimization, ethics (reduced use of animals), safety, and development programmes are achievable.

References

Adolph, E. F. (1949) Quantitative relations in the physiological contributions of mammals. *Science* **109**: 579–85

Ahmad, A. B., Bennett, P. N., Rowland, M. (1983) Models of hepatic drug clearance: discrimination between the 'well-stirred' and 'parallel-tube' models. *J. Pharm. Pharmacol*. **35**: 219–224

Andersson, T. B., Sjöberg, H., Hoffmann, K.-J., Boobis, A. R., Watts, P., Edwards, R. J., Lake, B. G., Price, R. J., Renwick, A. B., Gómez-Lechón, M. J., Castell, J. V., Ingelman-Sundberg, M., Hidestrand, M., Goldfarb, P. S., Lewis, D. F. V., Corcos, L. Guillouzo, A., Taavitsainin, P., Pelkonen, O. (2001) An assessment of human liver-derived *in vitro* systems to predict the *in vivo* metabolism and clearance of almokalant. *Drug Metab. Dispos*. **29**: 712–720

Andersson, T. B., Bredberg, E., Ericsson, H., Sjöberg, H. (2004) An evaluation of the *in vitro* metabolism data for predicting the clearance and drug-drug interaction potential of CYP2C9 substrates. *Drug Metab. Dispos*. **32**: 715–721

Anissimov, Y. G., Roberts, M. S. (2002) A compartmental model of hepatic disposition kinetics: 1. Model development and application to linear kinetics. *J. Pharmacokinet.. Pharmacodyn*. **29**: 131–156

Anissimov, Y. G., Bracken, A. J., Roberts, M. S. (1999) Interconnected-tubes model of hepatic elimination: Steady-state considerations. *J. Theor. Biol*. **199**: 435–447

Ashforth, E. I., Carlile, D. J., Chenery, R., Houston, J. B. (1995) Prediction of in vivo disposition from in vitro systems: clearance of phenytoin and tolbutamide using rat hepatic microsomal and hepatocyte data. *J. Pharmacol. Exp. Ther*. **274**: 761–766

Austin, R. P., Barton, P., Cockcroft, S. L., Wenlock, M. C., Riley, R. J. (2002) The influence of nonspecific microsomal binding on apparent intrinsic clearance and its prediction from physicochemical properties. *Drug Metab. Dispos.* **30**: 1497–1503

Austin, R. P., Barton, P., Mohmed, S., Riley, R. J. (2005) The binding of drugs to hepatocytes and its relationship to physicochemical properties. *Drug Metab. Dispos*. **33**: 419–425

Bäärnhielm, C., Dahlbäck, H., Skånberg, I. (1986) In vivo pharmacokinetics of felodipine predicted from in vitro studies in rat, dog and man. *Acta Pharmacol. Toxicol*. **59**: 113–122

Bachmann, K. (1989) Predicting toxicokinetic parameters in humans from toxicokinetic data acquired from three small mammalian species. *J. Applied Toxicol*. **9**: 331–338

Bachmann, K., Byers, J., Ghosh, R. (2003) Prediction of in vivo clearance from in vitro data using cryopreserved human hepatocytes. *Xenobiotica* **33**: 475–483

Bae, S. K., Lee, S. J., Kim, Y. G., Kim, S. H., Kim, J. W., Kim, T., Lee, M. G. (2005) Interspecies pharmacokinetic scaling of oltipraz in mice, rats, rabbits and dogs, and prediction of human pharmacokinetics. *Biopharm. Drug Dispos*. **26**: 99–115

Balakin, K. V., Ekins, S., Bugrim, A., Ivanenkov, Y. A., Korolev, D., Nikolsky, Y. V., Ivashchenko, A. A., Savchuk, N. P., Nikolskaya, T. (2004) Quantitative structure-metabolism relationship modeling of metabolic *N*-dealkylation reaction rates. *Drug Metab. Dispos*. **32**: 1111–1120

Balon, K., Riebesehl, B. U., Muller, B. W. (1999) Drug liposome partitioning as a tool for the prediction of human passive intestinal absorption. *Pharm. Res*. **16**: 882–888

Bayliss, M. K., Bell, J. A., Jenner, W. N., Park, G. R., Wilson, K. (1999) Utility of hepatocytes to model species differences in the metabolism of loxtidine and to predict pharmacokinetic parameters in rat, dog and man. *Xenobiotica* **29**: 253–268

Benedict, F. G. (1938) Vita energetics: a study in comparative basal metabolism. *Carnegie Inst. Publ*. **503**: 1–215

Björkman, S., Redke, F. (2000) Clearance of fentanyl, alfentanil, methohexitone, thiopentone and ketamine in relation to estimated hepatic blood flow in several animal species: application to prediction of clearance in man. *J. Pharm. Pharmacol*. **52**: 1065–1074

Blanchard, N., Richert, L. Notter, B., Delobel, F., David, P., Coassolo, P., Lavé, T. (2004) Impact of serum on clearance predictions obtained from suspensions and primary cultures of rat hepatocytes. *Eur. J. Pharm. Sci*. **23**: 189–199

Blanchard, N., Alexandre, E., Abadie, C., Lavé, T., Heyd, B., Mantion, G., Jaeck, D., Richert, L., Coassolo, P. (2005) Comparison of clearance predictions using primary cultures and suspensions of human hepatocytes. *Xenobiotica* **35**: 1–15

Blanchard, N., Hewitt, N., Silber, P., Jones, H. Coassolo, P., Lavé, T. (2006) Prediction of hepatic clearance using cryopreserved human hepatocytes: a comparison of serum and serum-free incubations. *J. Pharm. Pharmacol*. **58**: 633–641

Blom, A., Scaf, A. H. J., Meijer, D. K. F. (1982) Hepatic drug transport in the rat: A comparison between isolated hepatocytes, the isolated perfused liver and the liver in vivo. *Biochem. Pharmacol*. **31**: 1553–1565

Blouin, A. (1977) Morphometry of liver sinusoidal cells. In: Wisse, E., Knook, D. L. (eds) *Kupffer cells and other liver sinusoidal cells*. Elsevier/North-Holland Biomedical Press, New York

Bogaards, J. J. P., Bertrand, M., Jackson, P., Oudshoorn, M. J., Weaver, R. J., van Bladeren, P. J., Walther, B. (2000) Determining the best animal model for human cytochrome P450 activities: a comparison of mouse, rat, rabbit, dog, micropig, monkey and man. *Xenobiotica* **30**: 1131–1152

Bonate, P. L., Howard, D. (2000) Critique of prospective allometric scaling: Does the emperor have clothes? *J. Clin. Pharmacol*. **40**: 335–340

Bonati, M., Latinit, A., Tognoni, G., Young, J. F., Garattini, S. (1984) Interspecies comparison of in vivo caffeine pharmacokinetics in man, monkey, rabbit, rat and mouse. *Drug Met. Rev*. **15**: 1355–1383

Boobis, A., Gundert-Remy, U., Kremers, P., Macheras, P., Pelkonen, O. (2002) In silico prediction of ADME and pharmacokinetics. Report of an expert meeting organized by COST B15. *Eur. J. Pharm. Sci*. **17**: 183–193

Boxenbaum, H. (1980) Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: Extrapolation of data to benzodiazepines and phenytoin. *J. Pharmacokinet. Biopharm*. **8**: 165–176

Boxenbaum, H. (1982) Interspecies scaling, allometry, physiological time, and ground plan of pharmacokinetics. *J. Pharmacokinet. Biopharm*. **10**: 201–227

Boxenbaum, H., Fertig, J. B. (1984) Scaling of antipyrine intrinsic clearance of unbound drug in 15 mammalian species. *Eur. J. Drug Metab. Pharmacokinet*. **9**: 177–183

Boxenbaum, H., Ronfeld, R. (1983) Interspecies pharmacokinetic scaling and the Dedrick plots. *Am. J. Physiol*. R768–774

Brandon, E., Raap, C., Meijerman, I., Beijnen, J., Schellens, J. (2003) An update on in vitro test methods in human hepatic drug biotransformation research: pros and cons. *Toxicol. Appl. Pharmacol*. **189**: 233–246

Brody, S. (1937) Relativity of physiologic time and physiologic weight. *Growth* **1**: 60–67

Brody, S. (1945) In: *Bioenergetics and growth with special reference to the efficiency complex in domestic animals*. Reinhold, New York

Bugrim, A., Nikolskaya, T., Nikolsky, Y. (2004) Early prediction of drug metabolism and toxicity: systems biology approach and modeling. *Drug Disc. Today* **9**: 127–135

Caldwell, J. (1981) The current status of attempts to predict species differences in drug metabolism. *Drug Metab. Rev*. **12**: 221–237

Caldwell, G. W., Masucci, J. A., Yan, Z., Hageman, W. (2004) Allometric scaling of pharmacokinetic parameters in drug discovery: can human CL, V_{ss} and t½ be predicted from in-vivo rat data? *Eur. J. Drug Metab. Pharmacokinet*. **29**: 133–143

Campbell, B. (1994) Can allometric interspecies scaling be used to predict human kinetics? *Drug Inf. J*. **28**: 235–245

Cao, X., Gibbs, S. T., Fang, L., Miller, H. A., Landowski, C. P., Shin, H.-C., Lennernäs, H., Zhong, Y., Amidon, G. L., Yu, L. X., Sun, D. (2006) Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model? *Pharm. Res*. **23**: 1675–1686

Carlile, D. J., Stevens, A. J., Ashforth, E. I. L, Waghela, D., Houston, J. B. (1998) In vivo clearance of ethoxycoumarin and its prediction from in vitro systems. *Drug Metab. Dispos*. **26**: 216–221

Carlile, D. J., Hakooz, N., Bayliss, M. K., Houston, J. B. (1999a) Microsomal prediction of *in vivo* clearance of CYP2C9 substrates. *Br. J. Clin. Pharmacol*. **47**: 625–635

Carlile, D. J., Hakooz, N., Houston, J. B. (1999b) Kinetics of drug metabolism in rat liver slices: IV. Comparison of ethoxycoumarin clearance by liver slices, isolated hepatocytes, and hepatic microsomes from rats pretreated with known modifiers of cytochrome P-450 activity. *Drug Metab. Dispos*. **27**: 526–532

Carrel, A. (1931) Physiological time. *Science* **74**: 618–621

Chandra, P., Brouwer, K. L. R. (2004) The complexities of hepatic drug transport: current knowledge and emerging concepts. *Pharm. Res*. **21**: 719–735

Chiba, M., Fujita, S., Suzuki, T. (1990) Pharmacokinetic correlation between in vitro hepatic microsomal enzyme kinetics and in vivo metabolism of imipramine and desipramine in rats. *J. Pharm. Sci*. **79**: 281–287

Chiou, W. L. (1984) A new model-independent physiological approach to study hepatic drug clearance and its applications. *Int. J. Clin. Pharmacol. Ther. Toxicol*. **22**: 577–590

Chiou, W. L., Barve, A. (1998) Linear correlation of the fraction of oral dose absorbed of 64 drugs between humans and rats. *Pharm. Res*. **15**: 1792–1795

Chiou, W. L., Buehler, P. W. (2002) Comparison of oral absorption and bioavailability of drugs between monkey and human. *Pharm. Res*. **19**: 868–874

Chiou, W. L., Choi, Y. M. (1995) Unbound total (plasma) clearance approach in interspecies pharmacokinetics correlation: theophylline-cimetidine interaction. *Pharm. Res*. **12**: 1238–1239

Chiou, W. L., Hsu, F. H. (1988) Correlation between unbound plasma clearances of fifteen extensively metabolized drugs between humans and rats. *Pharm. Res*. **5**: 668–672

Chiou, W. L., Robbie, G., Chung, S. M., Wu, T. C., Ma, C. (1998) Correlation of plasma clearance of 54 extensively metabolized drugs between humans and rats: mean allometric coefficient of 0.66. *Pharm. Res*. **15**: 1474–1479

Chiou, W. L., Jeong, H. Y., Chung, S. M., Wu, T. C. (2000) Evaluation of using dog as an animal model to study the fraction of oral dose absorbed of 43 drugs in humans. *Pharm. Res*. **17**: 135–140

Chow, F. S., Piekoszewski, W., Jusko, W. J. (1997) Effect of hematocrit and albumin concentration on hepatic clearance of tacrolimus (FK506) during rabbit liver perfusion. *Drug Metab. Dispos*. **25**: 610–616

Chung, M., Radwanski, E., Loenberg, D., Lin, C., Oden, E., Symchowicz, S., Gural, R. P., Miller, G. H. (1985) Interspecies pharmacokinetic scaling of SCH34343. *J. Antimicrob. Chemother*. **15**: 227–233

Clarke, S. E., Jeffrey, P. (2001) Utility of metabolic stability screening: comparison of *in vitro* and *in vivo* clearance. *Xenobiotica* **31**: 591–598

Crivori, P., Zamora, I., Speed, B., Orrenius, C., Poggesi, I. (2004) Model based on GRID-derived descriptors for estimating CYP450 enzyme stability of potential drug candidates. *J Comput. Aided Mol. Design* **18**: 155–166

Cross, D. M., Bayliss, M. K. (2000) A commentary on the use of hepatocytes in drug metabolism studies during drug discovery and development. *Drug Metab. Rev*. **32**: 219–240

Cruze, C. A., Kelm, G. R., Meredith, M. P. (1995) Interspecies scaling of tebufelone pharmacokinetic data and application to preclinical toxicology. *Pharm. Res*. **12**: 895–901

Davidson, I. W. F., Parker, J. C., Beliles, R. P. (1986) Biological basis for extrapolation across mammalian species. *Reg. Toxicol. Pharmacol*. **6**: 211–237

Davies, B., Morris, T. (1993) Physiological parameters in laboratory animals and humans. *Pharm. Res*. **10**: 1093–1099

Davis, A. M., Riley, R. J. (2004) Predictive ADMET studies, the challenges and the opportunities. *Curr. Opin. Chem. Biol*. **8**: 378–386

De Graaf, I. A. M., van Meijeren, C. E., Pektas, F., Koster, H. J. (2002) Comparison of in vitro preparations for semi-quantitative prediction of in vivo drug metabolism. *Drug Metab. Dispos*. **30**: 1129–1136

De Kanter, R., Monshouwer, M., Draaisma, A. L., De Jager, M. H., De Graaf, A. M., Proost, J. H., Meijer, D. K. F., Groothuis, G. M. M. (2004) Prediction of whole-body metabolic clearance of drugs through the combined use of slices from rat liver, lung, kidney, small intestine and colon. *Xenobiotica* **34**: 229–241

De Loecker, W., Koptelov, V. A., Grischenko, V. I., De Loecker, P. (1998) Effects of cell concentration on viability and metabolic activity during cryopreservation. *Cryobiology* **37**: 103–109

Dedrick, R. L. (1973) Animal scale-up. *J. Pharmacokinet. Biopharm*. **1**: 435–461

Dedrick, R. L., Bischoff, K. B., Zaharko, D. S. (1970) Interspecies correlation of plasma concentration history of methotrexate (NSC-740). *Cancer Chemother. Rep. Part I* **54**: 95–101

Donato, M. T., Castell, J. V. (2003) Strategies and molecular probes to investigate the role of cytochrome P450 in drug metabolism. *Clin. Pharmacokinet*. **42**: 153–178

Donato, M. T., Gómez-Lechón, M. J., Castell, J. V. (1993) A micro-assay for measuring cytochrome P450IA1 and P450IIB1 activities in intact human and rat hepatocytes cultured on 96-well plates. *Anal. Biochem*. **213**: 29–33

Ekins, S. (2003) *In silico* approaches to predicting drug metabolism, toxicology and beyond. *Biochem. Soc. Transact*. **31**: 611–614

Ekins, S., Obach, S. R. (2000) Three-dimensional quantitative structure activity relationship computational approaches for prediction of human *in vitro* intrinsic clearance. *J. Pharmacol. Exp. Ther*. **295**: 463–473

Ekins, S., Rose, J. (2002) *In silico* ADME/Tox: The state of the art. *J. Mol. Graph. Mod*. **20**: 305–309

Ekins, S., Murray, G. I., Burke, M. D., Williams, J. A., Marchant, N. C., Hawksworth, G. M. (1995) Quantitative differences in phase I and II metabolism between rat precision-cut liver slices and isolated hepatocytes. *Drug Metab. Dispos*. **23**: 1274–1279

Ekins, S., Waller, C. L., Swaan, P. W., Cruciani, G., Wrighton, S. A., Wikel, J. H. (2000) Progress in predicting human ADME parameters in silico. *J. Pharmacol. Toxicol. Methods* **44**: 251–272

Ekins, S., Boulanger, B., Swaan, P. W., Hupcey, M. A. Z. (2002) Towards a new age of virtual ADME/TOX and multidimensional drug discovery. *J. Comput. Aided Mol. Design* **16**: 381–401

Ekins, S., Stresser, D. M., Williams, J. A. (2003) In vitro and pharmacophore insights into CYP3A enzymes. *Trends Pharmacol. Sci*. **24**: 161–166

Ethell, B. T., Ekins, S., Wang, J., Burchell, B. (2002) Quantitative structure activity relationships for the glucuronidation of simple phenols by expressed human UGT1A6 and UGT1A9. *Drug Metab. Dispos*. **30**: 734–738

Evans, A. M., Hussein, Z., Rowland, M. (1993) Influence on the distribution and elimination kinetics of diclofenac in the isolated perfused rat liver: analysis by the impulse-response technique and the dispersion model. *J. Pharm. Sci*. **82**: 421–428

Fabre, G., Combalbert, J., Berger, Y., Cano, J.-P. (1990) Human hepatocytes as a key in vitro model to improve preclinical drug development. *Eur. J. Drug Metab. Pharmacokinet*. **15**: 165–171

Fagerholm, U., Björnsson, M. A. (2005) Clinical pharmacokinetics of the cyclooxygenase-inhibiting nitric oxide donator (CINOD) AZD3582. *J. Pharm. Pharmacol*. **57**: 1539–1554

FDA. Guidance for industry and reviewers. Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers (Draft). *http://www.fda.gov/cder/guidance/3814dft.pdf*

Feng, M. R., Lou, X., Brown, R. R., Hutchaleelaha, A. (2000) Allometric pharmacokinetic scaling: towards the prediction of human oral pharmacokinetics. *Pharm. Res*. **17**: 410–418

Fischer, R. (1966) In: Fraser, J. T. (ed.) *The voices of time*. George Braziller, New York, pp 357–382

Floby, E., Briem, S., Terelius, Y., Sohlenius-Sternbeck, A.-K. (2004) Use of a cocktail of probe substrates for drug-metabolizing enzymes for the assessment of the metabolic capacity of hepatocyte preparations. *Xenobiotica* **34**: 949–959

Forker, E. L., Luxon, B. A. (1982) Hepatic transport kinetics: effect of anatomic and metabolic heterogenecity on estimates of the average transfer coefficients. *Am. J. Physiol*. **243**: G532–540

Gagner Milchert, I., Gabrielsson, J., Linde, J., Terelius, Y. (1998) In vitro-in vivo extrapolation of robalzotan metabolic parameters: an interspecies comparison. *Eur. Neuropsychopharmacol*. **8**: 184–185

Galetin, A., Brown, C., Hallifax, D., Ito, K., Houston, J. B. (2004) Utility of recombinant enzyme kinetics in prediction of human clearance: impact of variability, CYP3A5, and CYP2C19 on CYP3A4 probe substrates. *Drug Metab. Dispos*. **32**: 1411–1420

Gascon, A. R., Calvo, B., Hernandez, R. M., Dominguez-Gil, A., Pedraz, J. L. (1994) Interspecies scaling of cimetidine-theophylline pharmacokinetic interaction: interspecies scaling in pharmacokinetic interactions. *Pharm. Res*. **11**: 945–950

Gebhardt, R. (1992) Metabolic zonation of the liver: regulation and implications for liver function. *Pharmacol. Ther*. **53**: 275–354

Glaeser, H., Drescher, S., Hofmann, U., Heinkele, G., Somogyi, A. A., Eichelbaum, M., Fromm, M. F. (2004) Impact of concentration and rate of intraluminal drug delivery on absorption and gut wall metabolism of verapamil in humans. *Clin. Pharmacol. Ther*. **76**: 230–238

Gobburu, J. V. S., Shelver, W. H. (1995) Quantitative structure-pharmacokinetic relationships (QSPR) of beta blockers derived using neural networks. *J. Pharm. Sci*. **84**: 862–865

Gómez-Lechón, M. J., Donato, M. T., Castell, J. V., Jover, R. (2003) Human hepatocytes as a tool for studying toxicity and drug metabolism. *Curr. Drug Metab*. **4**: 292–312

Gómez-Lechón, M. J., Donato, M. T., Castell, J. V., Jover, R. (2004) Human hepatocytes in primary culture: the choice to investigate drug metabolism in man. *Curr. Drug Metab*. **5**: 443–462

Goodman Gilman, A. (2001) In: Hardman, J. G., Limbird, L. E., Gilman, A. G. (eds) *Goodman and Gilman's: The pharmacological basis of therapeutics*. 10th edn, int. edn, McGraw-Hill

Goresky, C. A., Pang, K. S., Schwab, A. J., Barker III, F., Cherry, W. F., Bach, G. G. (1992) Uptake of a protein-bound polar compound, acetaminophen sulphate, by perfused rat liver. *Hepatology* **16**: 173–190

Goresky, C. A., Bach, G. G., Simard, A., Schwab, A. J., Bracht, A. (2000) Uptake of lactate by the liver: effect of red blood cell carriage. *Am. J. Physiol. Gastroint. Liver Physiol*. **278**: G775–788

Grene-Lerouge, N., Bazin-Redureau, M., Debray, M., Scherrmann, J. (1996) Interspecies scaling of clearance and volume of distribution for digoxin-specific fab. *Toxicol. Appl. Pharmacol*. **138**: 84–89

Griffin, S. J., Houston, J. B. (2004) Comparison of fresh and cryopreserved rat hepatocyte suspensions for the prediction of *in vitro* intrinsic clearance. *Drug Metab. Dispos*. **32**: 552–558

Griffin, S. J., Houston, J. B. (2005) Prediction of *in vitro* intrinsic clearance from hepatocytes: comparison of suspension and monolayer cultures. *Drug Metab. Dispos*. **33**: 115–120

Grime, K., Riley, R. J. (2006) The impact of in vitro binding on in vitro–in vivo extrapolations, projections of metabolic clearance and clinical drug-drug interactions. *Curr. Drug Metab*. **7**: 251–264

Groothuis, G. M., Meijer, D. K. (1996) Drug traffic in the hepatobiliary system. *J. Hepatol*. **24**: 3–28

Günter, B. (1975) On theories of biological similarity. In: *Beier, W. (Hrsg.) Fortschritte der experimentellen und theoretischen biophysic*. Bd. 19. Edition Leipzig, Leipzig

Haddad, S., Funk, C. (2001) A novel liver PBPK model as tool for *in vitro-in vivo* extrapolations. *Drug Metab. Rev*. **33**(Suppl. 1): 204

Haenen, B., Rompelberg, C., van Twillert, K., Hamzink, M., Dormans, J., van Eijkeren, J. (2002) Utility of rat liver slices to estimate rat hepatic clearance for application in physiologically based pharmacokinetic modeling: A study with tolbutamide, a compound with low extraction efficiency. *Drug Metab. Dispos*. **30**: 307–313

Hakooz, N., Ito, K., Rawden, H., Gill, H., Lemmers, L., Boobis, A. R., Edwards, R. J., Carlile, D. J., Lake, B. G., Houston, J. B. (2006) Determination of a human hepatic microsomal scaling factor for predicting in vivo drug clearance. *Pharm. Res*. **23**: 533–539

Hallifax, D., Rawden, H. C., Hakooz, N., Houston, J. B. (2005) Prediction of metabolic clearance using cryopreserved human hepatocytes: kinetic characteristics for five benzodiazepines. *Drug Metab. Dispos*. **33**: 1852–1858

Hansch, C., Leo, A., Mekapati, S. B., Kurup, A. (2004) QSAR and ADME. *Bioorg. Med. Chem*. **12**: 3391–3400

Hayes, K. A., Brennan, B., Chenery, R., Houston, J. B. (1995) *In vivo* disposition of caffeine predicted from hepatic microsomal and hepatocyte data. *Drug Metab. Dispos*. **23**: 349–353

Hayssen, V., Lacy, R. C. (1985) Basal metabolic rates in mammals: taxonomic differences in the allometry of BMR and body mass. *Comp. Biochem. Physiol*. **81**: 741–754

Hayton, W. L. (1989) Pharmacokinetic parameters for interspecies scaling using allometric techniques. *Hlth. Phys*. **57** (Suppl. 1): 159–164

Henderson, A. J., Guzzo, P. R. (2004) Metabolism-driven optimization of pharmacokinetics. *Curr. Drug Disc*. **May**: 17–22

Hengstler, J. G., Utesch, D., Steinberg, P., Platt, K. L., Diener, B., Ringel, M., Swales, N., Fischer, T., Biefang, K., Gerl, M., Bottger, T., Oesch, F. (2000) Cryopreserved primary hepatocytes as a constantly available in vitro model for the evaluation of human and animal drug metabolism and enzyme induction. *Drug Metab. Rev*. **32**: 81–118

Hewitt, N. J., Bühring, K.-U., Dasenbrock, J., Haunschild, J., Ladstetter, B., Utesch, D. (2001) Studies comparing in vivo:in vitro metabolism of three pharmaceutical compounds in rat, dog, monkey, and human using cryopreserved hepatocytes, microsomes, and collagen gel immobilized hepatocyte cultures. *Drug Metab. Dispos*. **29**: 1042–1050

Hinderling, P. H., Dilea, C., Koziol, T., Millington, G. (1993) Comparative kinetics of sematilide in four species. *Drug Metab. Dispos*. **21**: 662–669

Hirota, N., Ito, K., Iwatsubo, T., Green, C. E., Tyson, C. A., Shimada, N., Suzuki, H., Sugiyama, Y. (2001) *In vitro/in vivo* scaling of alprazolam metabolism by CYP3A4 and CYP3A5 in humans. *Biopharm. Drug Dispos*. **22**: 53–71

Hoener, B.-A. (1994) Predicting the hepatic clearance of xenobiotics in humans from in vitro data. *Biopharm. Drug Dispos*. **15**: 295–304

Houle, R., Raoul, J., Lévesque, J.-F., Pang, K. S., Nicoll-Griffith, D. A., Silva, J. M. (2003) Retention of transporter activities in cryopreserved, isolated rat hepatocytes. *Drug Metab. Dispos*. **31**: 447–451

Houston, J. B. (1994) Utility of *in vitro* drug metabolism data in predicting *in vivo* metabolic clearance. *Biochem. Pharmacol*. **47**: 1469–1479

Houston, J. B., Carlile, D. J. (1997) Prediction of hepatic clearance from microsomes, hepatocytes, and liver slices. *Drug Metab. Rev*. **29**: 891–922

Hu, T.-M., Hayton, W. L. (2001) Allometric scaling of xenobiotic clearance: uncertainty and universality. *AAPS Pharm. Sci*. **3**: article 29

Hung, D. Y., Mellick, G. D., Anissimov, Y. G., Weiss, M., Roberts, M. S. (1998) Hepatic structure-pharmacokinetic relationships: The hepatic disposition and metabolite kinetics of a homologous series of *O*-acyl derivatives of salicylic acid. *Br. J. Pharmacol*. **124**: 1475–1483

Hung, D. Y., Chang, P, Weiss, M., Roberts, M. S. (2001) Structure-hepatic disposition relationship for cationic drugs in isolated perfused rat livers: transmembrane exchange and cytoplasmic binding process. *J. Pharmacol. Exp. Ther*. **297**: 780–789

Hung, D. Y., Siebert, G. A., Chang, P., Anissimov, Y. G., Roberts, M. S. (2004) Disposition kinetics of propranolol isomers in the perfused rat liver. *J. Pharmacol. Exp. Ther*. **311**: 822–829

Hussain, A. S., Johnson, R. D., Vachharajani, N. N., Ritschel, W. A. (1993a) Feasibility of developing a neural network for prediction of human pharmacokinetic parameters from animal data. *Pharm. Res*. **10**: 466–469

Hussein, Z., Evans, A. M., Rowland, M. (1993b) Physiological models of hepatic drug clearance: Influence of altered protein binding on the elimination of diclofenac in the isolated perfused rat liver. *J. Pharm. Sci*. **82**: 880–885

Igari, Y., Sugiyama, Y., Sawada, Y., Iga, T., Hanano, M. (1984) In vitro and in vivo assessment of hepatic and extrahepatic metabolism of diazepam in the rat. *J. Pharm. Sci*. **73**: 826–828

Ings, R. M. (1990) Interspecies scaling and comparisons in drug development and toxicokinetics. *Xenobiotica* **20**: 1201–1231

Ito, K., Houston, J. B. (2004) Comparison of the use of liver models for predicting drug clearance using in vitro kinetic data from hepatic microsomes and isolated hepatocytes. *Pharm. Res*. **21**: 785–792

Ito, K., Houston, J. B. (2005) Prediction of human drug clearance from in vitro and preclinical data using physiologically based and empirical approaches. *Pharm. Res*. **22**: 103–112

Ito, K., Iwatsubo, T., Kanamitsu, S., Nakajima, Y., Sugiyama, Y. (1998) Quantitative prediction of in vivo drug clearance and drug interactions from in vitro data on metabolism, together with binding and transport. *Annu. Rev. Pharmacol. Toxicol*. **38**: 461–499

Iwatsubo, T., Hirota, N., Ooie, T., Suzuki H., Sugiyama Y. (1996) Prediction of in vivo drug disposition from in vitro data based on physiological pharmacokinetics. *Biopharm. Drug Disp*. **17**: 273–310

Iwatsubo, T., Hirota, N., Ooie, T., Suzuki, H., Shimada, N., Chiba, K., Ishizaki, T., Green, C. E., Tyson, C. A., Sugiyama, Y. (1997) Prediction of *in vivo* drug metabolism in the human liver from in vitro metabolism data. *Pharmacol. Ther*. **73**: 147–171

Izumi, T., Hosiyama, K., Enomoto, S., Sasahara, K., Sugiyama, Y. (1997) Pharmacokinetics of troglitazone, an antidiabetic agent: Prediction of in vivo stereoselective sulfation and glucuronidation from *in vitro* data. *J. Pharmacol. Exp. Ther*. **280**: 1392–1400

Jansen, J. A. (1981) Influence of plasma protein binding kinetics on hepatic clearance assessed from a "tube" model and a "well-stirred" model. *J. Pharmacokinet. Biopharm*. **9**: 15–26

Jezequel, S. G. (1994) Fluconazole: Interspecies scaling and allometric relationships of pharmacokinetic properties. *J. Pharm. Pharmacol*. **46**: 196–199

Jolivette, L. J., Ward, K. W. (2005) Extrapolation of human pharmacokinetic parameters from rat, dog, and monkey data: molecular properties associated with extrapolative success or failure. *J. Pharm. Sci*. **94**: 1467–1483

Kansy, M., Senner, F., Gubernator, K. (1998) Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. *J. Med. Chem*. **41**: 1007–1010

Kern, A., Bader, A., Pichlmayr, R., Sewing, K.-F. (1997) Drug metabolism in hepatocyte sandwich cultures of rats and humans. *Biochem. Pharmacol*. **54**: 761–772

Kimura, T., Iwasaki, N., Yokoe, J.-I., Haruta, S., Yokoo, Y., Ogawara, K.-I., Higaki, K. (2000) Analysis and prediction of absorption profile including hepatic first-pass metabolism of *N*-methyltyramine, a potent stimulant of gastrin release present in beer, after oral ingestion in rats by gastrointestinal-transit-absorption model. *Drug Metab. Dispos*. **28**: 577–581

Kleiber, M. (1932) Body size and metabolism. *Hilgardia* **6**: 315–332

Kleiber, M. (1961) In: *The fire of life: an introduction to animal energetics*. Wiley, New York

Kleiber, M. (1975) Metabolic turnover rate: A physiological meaning of the metabolic rate per unit body weight. *J. Theor. Biol*. **53**: 199–204

Klotz, U., Antonin, K.-H., Beck, P. R. (1976) Pharmacokinetics and plasma binding of diazepam in man, dog, rabbit, guinea pig and rat. *J. Pharmacol. Exp. Ther*. **199**: 67–73

Kono, Y., Yang, S., Roberts, E. A. (1997) Extended primary culture of human hepatocytes in a collagen gel sandwich system. *In Vitro Cell. Dev. Biol. Anim*. **33**: 467–472

Krasovskij, G. N. (1976) Extrapolation of experimental data from animals to man. *Environ. Health Prespect*. **13**: 51–58

Kroemer, H. K., Echizen, H., Heidemann, H., Eichelbaum, M. (1992) Predictability of the in vivo metabolism of verapamil from in vitro data: contribution of individual metabolic pathways and stereoselective aspects. *J. Pharmacol. Exp. Ther*. **260**: 1052–1057

Kumar, S., Samuel, K., Subramanian, R., Braun, M. P., Stearns, R. A., Chiu, S. H., Evans, D. C., Baillie, T. A. (2002) Extrapolation of diclofenac clearance from in vitro microsomal metabolism data: role of acyl glucuronidation and sequential oxidative metabolism of the acyl glucuronide. *J. Pharmacol. Exp. Ther*. **303**: 969–978

Kwon, Y., Morris, M. E. (1997a) Membrane transport in hepatic clearance of drugs I: Extended hepatic clearance models incorporating concentration-dependent transport and elimination processes. *Pharm. Res*. **14**: 774–779

Kwon, Y., Morris, M. E. (1997b) Membrane transport in hepatic clearance of drugs II: Zonal distribution patterns of concentration-dependent transport and elimination processes. *Pharm. Res*. **14**: 780–785

Lam, J. L., Benet, L. Z. (2004) Hepatic microsome studies are insufficient to characterize in vivo hepatic metabolic clearance and metabolic drug-drug interactions: studies of digoxin metabolism in primary rat hepatocytes vs microsomes. *Drug Metab. Dispos*. **32**: 1311–1316

Lapka, R., Rejholec, V., Sechser, T., Peterkova, M., Smid M. (1989) Interspecies pharmacokinetic scaling of metazosin, a novel alpha-adrenergic antagonist. *Biopharm. Drug Dispos*. **10**: 581–589

Lau, Y. Y., Sapidou, E., Cui, X., White, R. E., Cheng, K.-C. (2002) Development of a novel in vitro model to predict hepatic clearance using fresh, cryopreserved, and sandwich-cultured hepatocytes. *Drug Metab. Dispos*. **30**: 1446–1454

Lavé, T., Coassolo, P. (1998) Commentary on "Integration of in vitro data and brain weight in allometric scaling to predict clearance in humans: Some suggestions". *J. Pharm. Sci*. **87**: 530

Lavé, T., Levet-Trafit, B., Schmitt-Hoffmann, A. H., Morgenroth, B., Richter, W., Chou, R. C. (1995a) Interspecies scaling of interferon disposition and comparison of allometric scaling with concentration-time transformations. *J. Pharm. Sci*. **84**: 1285–1290

Lavé, T., Schmitt-Hoffmann, A. H., Coassolo, P., Valles, B., Ubeaud, G., Ba, B., Brandt, R., Chou, R. C. (1995b) A new extrapolation method from animals to man: application to a metabolized compound, mofarotene. *Life Sci*. **56**: 473–478

Lavé, T., Dupin, S., Schmitt, M., Kapps, M., Meyer, J., Morgenroth, B., Chou, R. C., Jaeck, D., Coassolo P. (1996a) Interspecies scaling of tolcapone, a new inhibitor of catechol-O-methyltransferase (COMT). Use of in vitro data from hepatocytes to predict metabolic clearance in animals and humans. *Xenobiotica* **26**: 839–851

Lavé, T., Coassolo, P., Ubeaud, G., Brandt, R., Schmitt, C., Dupin, S., Jaeck, D., Chou, R. C. (1996b) Interspecies scaling of bosentan, a new endothelin receptor antagonist and integration of in vitro data into allometric scaling. *Pharm. Res*. **13**: 97–101

Lavé, T., Saner, A., Coassolo, P., Brandt, R., Schmitt-Hoffmann, A. H., Chou, R. C. (1996c) Animal pharmacokinetics and interspecies scaling from animals to man of lamifiban, a new platelet aggregation inhibitor. *J. Pharm. Pharmacol*. **48**: 573–577

Lavé, T., Dupin, S., Schmitt, C., Chou, R. C., Jaeck, D., Coassolo, P. (1997a) Integration of *in vitro* data into allometric scaling to predict hepatic metabolic clearance in man: application to 10 extensively metabolized drugs. *J. Pharm. Sci*. **86**: 584–590

Lavé, T., Dupin, S., Schmitt, C., Valles, B., Ubeaud, G., Chou, R. C., Jaeck, D., Coassolo, P. (1997b) The use of human hepatocytes to select compounds based on their expected hepatic extraction ratios in humans. *Pharm. Res*. **14**: 152–155

Lavé, T., Coassolo, P., Reigner, B. (1999a) Prediction of hepatic metabolic clearance based on interspecies scaling techniques and in vitro-in vivo correlations. *Clin. Pharmacokinet*. **36**: 211–231

Lavé, T., Portmann, R., Schenker, G., Gianni, A., Guenzi, A., Girometta, M.-A., Schmitt, M. (1999b) Interspecies pharmacokinetic comparisons and allometric scaling of napsagatran, a low molecular weight thrombin inhibitor. *J. Pharm. Pharmacol*. **51**: 85–91

Le Vee, M., Jigorel, E., Glaise, D., Gripon, P., Guguen-Guillouzo, C., Fardel, O. (2006) Functional expression of sinusoidal and canalicular hepatic drug transporters in the differentiated human hepatoma HepaRG cell line. *Eur. J. Pharm. Sci*. **28**: 109–117

LeCluyse, E. L., Bullock, P. L., Parkinson, A. (1996) Strategies for restoration and maintenance of normal hepatic structure and function in long-term cultures of rat hepatocytes. *Adv. Drug Del. Rev*. **22**: 133–186

Lee, H. L., Chiou, W. L. (1989a) Erythrocytes as barriers for drug elimination in the isolated liver: I. Doxorubicin. *Pharm. Res*. **6**: 833–839

Lee, H. L., Chiou, W. L. (1989b) Erythrocytes as barriers for drug elimination in the isolated liver: II. Propranolol. *Pharm. Res*. **6**: 840–843

Leggett, R. W., Williams, L. R. (1995) A proposed blood circulation model for reference man. *Health Physics* **69**: 187–201

Lennernäs, H. (1997) Human jejunal effective permeability and its correlation with preclinical drug absorption models. *J. Pharm. Pharmacol*. **49**: 627–638

Lepist, E.-I., Jusko, W. J. (2004) Modeling and allometric scaling of s(+)-ketoprofen pharmacokinetics and pharmacodynamics: a retrospective analysis. *J. Vet. Pharmacol. Ther*. **27**: 211–218

Lester, D., Keokosky, W. Z. (1967) Alcohol metabolism in the horse. *Life Sci*. **6**: 2313–2319

Li, A. P., Lu, C., Brent, J. A., Pham, C., Fackett, A., Ruegg, C. E., Silber, P. M. (1999) Cryopreserved human hepatocytes: characterization of drug-metabolising enzyme activities and applications in higher throughput screening assays for hepatotoxicity, metabolic stability and drug-drug interaction potential. *Chem. Biol. Interact*. **121**: 17–35

Lin, J. H. (1995) Species similarities and differences in pharmacokinetics. *Drug Metab. Dispos*. **23**: 1008–1021

Lin, J. H., Hayashi, M., Awazu, S., Hanano, M. (1978) Correlation between in vitro and in vivo drug metabolism rate: oxidation of ethoxybenzamide in rat. *J. Pharmacokinet. Biopharm*. **6**: 327–337

Lindstedt, S. L., Schaeffer, P. J. (2002) Use of allometry in predicting anatomical and physiological parameters of mammals. *Lab. Animals* **36**: 1–19

Liu, L., Pang, K. S. (2005) The roles of transporters and enzymes in hepatic drug processing. *Drug Metab. Dispos*. **33**: 1–9

Liu, X., LeCluyse, E. L., Brouwer, K. R., Lightfoot, R. M., Lee, J. I., Brouwer, K. L. (1999) Use of Ca^{2+} modulation to evaluate biliary excretion in sandwich-cultured rat hepatocytes. *J. Pharmacol. Exp. Ther*. **289**: 1592–1599

Liu, X. D., Chen, J. (2001) Prediction of drug clearance in humans from laboratory animals based on body surface area. *Eur. J. Drug Metab. Pharmacokinet*. **26**: 249–256

Lombardo, F., Gifford, E., Shalaeva, M. Y. (2003) In silico ADME prediction: data, models, facts and myths. *Mini Rev. Med. Chem*. **3**: 861–875

Luttringer, O., Theil, F.-P., Poulin, P., Schmitt-Hoffmann, A. H., Guentert, T. W., Lavé, T. (2003) Physiologically based pharmacokinetic (PBPK) modelling of disposition of epiroprim in humans. *J. Pharm. Sci*. **92**: 1990–2007

MacGregor, J. T., Collins, J. M., Sugiyama, Y., Tyson, C. A., Dean, J., Smith, L., Andersen, M. E., Curren, R. D., Houston, J. B., Kadlubar, F. F., Kedderis, G. L., Krishnan, K., Li, A. P., Parchment, R. E., Thummel, K., Tomaszewski, J. E., Ulrich, R.,

Vickers, A. E., Wrighton, S. A. (2001) In vitro human tissue models in risk assessment: report of a consensus-building workshop. *Toxicol. Sci*. **29**: 17–36

Mahmood, I. (1997) Prediction of absolute bioavailability for drugs using oral and renal clearance following a single oral dose: A critical review. *Biopharm. Drug Dispos*. **18**: 465–473

Mahmood, I. (1998) Integration of in vitro data and brain weight in allometric scaling to predict clearance in humans: some suggestions. *J. Pharm. Sci*. **87**: 527–529

Mahmood, I. (1999) Prediction of clearance, volume of distribution and half-life by allometric scaling and by use of plasma concentrations predicted from pharmacokinetic constants: a comparative study. *J. Pharm. Pharmacol*. **51**: 905–910

Mahmood, I. (2001) Interspecies scaling: is a priori knowledge of cytochrome P450 isozymes involved in drug metabolism helpful in prediction of clearance in humans from animal data? *Drug Metab. Drug Interact*. **18**: 135–147

Mahmood, I. (2002a) Interspecies scaling: Predicting oral clearance in humans. *Am. J. Ther*. **9**: 35–42

Mahmood, I. (2002b) Prediction of clearance in humans from in vitro human liver microsomes and allometric scaling. A comparative study of the two approaches. *Drug Metab. Drug Interact*. **19**: 49–64

Mahmood, I. (2004) Interspecies scaling of protein drugs: prediction of clearance from animals to humans. *J. Pharm. Sci*. **93**: 177–185

Mahmood, I. (2005) The correction factors do help in improving the prediction of human clearance from animal data. *J. Pharm. Sci*. **94**: 940–947

Mahmood, I., Balian, J. D. (1996a) Interspecies scaling: predicting pharmacokinetic parameters of antiepileptic drugs in humans from animals with special emphasis on clearance. *J. Pharm. Sci*. **85**: 411–414

Mahmood, I., Balian, J. D. (1996b) Interspecies scaling: a comparative study for the prediction of clearance and volume using two or more than two species. *Life Sci*. **59**: 579–585

Mahmood, I., Balian, J. D. (1996c) Interspecies scaling: predicting clearance of drugs in humans. Three different approaches. *Xenobiotica* **26**: 887–895

Mahmood, I., Balian, J. D. (1999) The pharmacokinetic principles behind scaling from preclinical results to phase I protocols. *Clin. Pharmacokinet*. **36**: 1–11

Mahmood, I., Green, M. D., Fisher, J. E. (2003) Selection of the first-time dose in humans: comparison of different approaches based on interspecies scaling of clearance. *J. Clin. Pharmacol*. **43**: 692–697

Masimirembwa, C. M., Bredberg, U., Andersson, T. B. (2003) Metabolic stability for drug discovery and development. Pharmacokinetic and biochemical challenges. *Clin. Pharmacokinet*. **42**: 515–528

Matsui, K., Taniguchi, S., Yoshimura, T. (1999) Correlation of the clearance of donepezil (Aricept®) between in vivo and in vitro studies in rat, dog and human. *Xenobiotica* **29**: 1059–1072

McGinnity, D. F., Soars, M. G, Urbanowicz, R. A., Riley, R. J. (2004) Evaluation of fresh and cryopreserved hepatocytes as in vitro drug metabolism tools for the prediction of metabolic clearance. *Drug Metab. Dispos*. **32**: 1247–1253

McMahon, T. A., Bonner, J. T. (1983) On size and life. *Sci. Am. Lib. New York*

Meier, P. J. Sztul, E. S., Reuben, A., Boyer, J. L. (1984) Structural and functional polarity of canalicular and basolateral plasma membrane vesicles isolated in high yield from rat liver. *J. Cell Biol*. **98**: 991–1000

Mellet, L. B. (1969) Comparative drug metabolism. *Prog. Drug Res*. **13**: 136–169

Mellick, G. D., Roberts, M. S. (1996) The disposition of aspirin and salicylic acid in the isolated perfused rat liver: The effect of normal and retrograde flow on availability and mean transit time. *J. Pharm. Pharmacol*. **48**: 738–743

Mellick, G. D., Roberts, M. S. (1999) Structure-hepatic disposition relationships for phenolic compounds. *Toxicol. Applied Pharmacol*. **158**: 50–60

Miyauchi, S., Sawada, Y., Iga, T., Hanano, M., Sugiyama, Y. (1993) Comparison of the hepatic uptake clearances of fifteen drugs with a wide range of membrane permeabilities in isolated rat hepatocytes and perfused rat livers. *Pharm. Res*. **10**: 434–440

Mohutsky, M. A., Chien, J. Y., Ring, B. J., Wrighton, S. A. (2006) Predictions of the in vivo clearance of drugs from rate of loss using human liver microsomes for phase I and phase II biotransformations. *Pharm. Res*. **23**: 654–662

Mordenti, J. (1985) Pharmacokinetic scale-up: accurate prediction of human pharmacokinetic profiles from animal data. *J. Pharm. Sci*. **74**: 1097–1099

Mordenti, J. (1986) Man vs beast: pharmacokinetic scaling in mammals. *J. Pharm. Sci*. **75**: 1028–1040

Mordenti, J., Chen, S. A., Moore, J. A., Ferraiolo, B. L., Green, J. D. (1991) Interspecies scaling of clearance and volume of distribution data for five therapeutic proteins. *Pharm. Res*. **8**: 1351–1359

Mordenti, J., Osaka, G., Garcia, K., Thomsen, K., Licko, V., Meng, G. (1996) Pharmacokinetics and interspecies scaling of recombinant human factor VIII. *Toxicol. Appl. Pharmacol*. **136**: 75–78

Nagilla, R., Ward, K. W. (2004) A comprehensive analysis of the role of correction factors in the allometric predictivity of clearance from rat, dog, and monkey to humans. *J. Pharm. Sci*. **93**: 2522–2534

Naritomi, Y., Terashita, S., Kimura, S., Suzuki, A., Kagayama, A., Sugiyama, Y. (2001) Prediction of human hepatic clearance from in vivo animal experiments and in vitro metabolic studies with liver microsomes from animals and humans. *Drug Metab. Dispos*. **29**: 1316–1324

Naritomi, Y., Terashita, S., Kagayama, A., Sugiyama, Y. (2003) Utility of hepatocytes in predicting drug metabolism: comparison of hepatic intrinsic clearance in rats and humans in vivo and in vitro. *Drug Metab. Dispos*. **31**: 580–588

Niro, R., Byers, J. P., Fournier, R. L., Bachmann, K. (2003) Application of a convective-dispersion model to predict in vivo hepatic clearance from in vitro measurements utilizing cryopreserved human hepatocytes. *Curr. Drug Metab*. **4**: 357–369

Obach, R. S. (1999) Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab. Dispos*. **27**: 1350–1359

Obach, R. S. (2000) Metabolism of ezlopitant, a nonpeptidic substance P receptor antagonist, in liver microsomes: Enzyme kinetics, cytochrome P450 isoform identity, and in vitro-in vivo correlation. *Drug Metab. Dispos*. **28**: 1069–1076

Obach, R. S. (2001) The prediction of human clearance from hepatic microsomal metabolism data. *Curr. Opin. Drug Disc. Develop*. **4**: 36–44

Obach, R. S., Reed-Hagen, A. E. (2002) Measurement of Michaelis constants for cytochrome P450-mediated biotransformation reactions using a substrate depletion approach. *Drug Metab. Dispos*. **30**: 831–837

Obach, R. S., Baxter, J. G., Liston, T. E., Silber, B. M., Jones, B. C., MacIntyre, F., Rance, D. J., Wastall, P. (1997) The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. *J. Pharmacol. Exp. Ther*. **283**: 46–58

Ott, P., Weisiger, R. A. (1999) Nontraditional effects of protein binding and hematocrit on uptake of indocyanine green by perfused rat liver. *Am. J. Physiol*. **273**: G227–238

Ott, P., Bass, L., Keiding, S. (1997) Hepatic ICG removal in the pig depends on plasma protein and hematocrit: Evidence of sinusoidal binding disequilibrium and unstirred water layer effects. *Hepatology* **26**: 679–690

Owens, S. M., Hardwick, W. C., Blackall, D. (1987) Phencyclidine pharmacokinetic scaling among species. *J. Pharmacol. Exp. Ther*. **242**: 96–101

Påhlman, I., Kankaanranta, S., Palmer L. (2001) Pharmacokinetics of tolterodine, a muscarinic receptor antagonist, in mouse, rat and dog. Interspecies relationship comparing with human pharmacokinetics. *Arzneimittelforschung* **51**: 134–144

Palm, K., Stenberg, P., Luthman, K., Artursson, P. (1997) Polar molecular surface properties predict the intestinal absorption of drugs in humans. *Pharm. Res*. **14**: 568–571

Pang, K. S., Rowland, M. (1977) Hepatic clearance of drugs I. Theoretical considerations of a "well-stirred" model and a "parallel tube" model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J. Pharmacokinet. Biopharm*. **5**: 625–653

Pang, K. S., Barker, F., Simard, A., Schwab, A. J., Goresky, C. A. (1995) Sulphation of acetaminophen by the perfused rat liver: the effect of red blood cell carriage. *Hepatology* **22**: 267–282

Pardridge, W. M., Sakiyama, R., Fierer, G. (1983) Transport of propranolol and lidocaine through the rat blood brain barrier. Primary role of globulin-bound drug. *J. Clin. Invest*. **71**: 900–908

Paxton, J. W., Kim, S. N., Whitfield, L. R. (1990) Pharmacokinetic and toxicity scaling of the antitumor agents amsacrine and CI-921, a new analogue, in mice, rats, rabbits, dogs, and humans. *Cancer Res*. **50**: 2692–2697

Penzotti, J. E., Landrum, G. A., Putta, S. (2004) Building predictive ADMET models for early decisions in drug discovery. *Curr. Opin. Drug Disc. Develop*. **7**: 49–61

Pérez, M. A. C., Snaz, M. B., Torres, L. R., Ávalos, R. G., González, M. P., Díaz, H. G. (2004) A topological sub-structural approach for predicting human intestinal absorption. *Eur. J. Med. Chem*. **39**: 905–916

Peters, R. H. (1983) *The ecological implications of body size*. Cambridge University Press, Cambridge.

Piekoszewski, W., Chow, F. S., Jusko, W. J. (1993) Disposition of tacrolimus (FK 506) in rabbits. Role of red blood cell binding in hepatic clearance. *Drug Metab. Dispos*. **21**: 690–698

Plant, N. (2004) Strategies for using *in vitro* screens in drug metabolism. *Drug Disc. Today* **9**: 328–336

Poggesi, I. (2004) Predicting human pharmacokinetics from preclinical data. *Curr. Opin. Drug Disc. Develop*. **7**: 100–111

Pond, S. M., Tozer, T. N. (1984) First-pass elimination. Basic concepts and clinical consequences. *Clin. Pharmacokinet*. **9**: 1–25

Proost, J. H., Nijssen, H. M. J., Strating, C. B., Meijer, D. K. F., Groothuis, G. M. M. (1993) Pharmacokinetic modeling of the sinusoidal efflux of anionic ligands from the isolated perfused rat liver: The influence of albumin. *J. Pharmacokinet. Biopharm*. **21**: 375–394

Puigdemont, A., Guitart, R., Mora, F., Arboix, M. (1991) Prediction of the disposition of propafenone in humans and dogs from pharmacokinetic parameters in other species. *J. Pharm. Sci*. **80**: 1106–1109

Railland, L., Guyomard, C., Scotte, M., Chesne, C., Guillouzo, A. (2000) Viability and drug metabolizing capacity of alginate-entrapped hepatocytes after cryopreservation. *Cell Biol. Toxicol*. **16**: 105–116

Rane, A., Wilkinson, G. R., Shand, D. G. (1977) Prediction of hepatic extraction ratiofrom *in vitro* measurement of intrinsic clearance. *J. Pharmacol. Exp. Ther*. **200**: 420–424

Reinoso, R. F, Telfer, B. A., Brennan, B. S., Rowland, M. (2001) Uptake of teicoplanin by isolated rat hepatocytes: Comparison with in vivo hepatic distribution. *Drug Metab. Dispos*. **29**: 453–459

Richter, W. F., Heizmann, P., Meyer, J., Starke, V., Lavé, T. (1998) Animal pharmacokinetics and interspecies scaling of Ro 25–6833 and related (lactamylvinyl)cephalosporins. *J. Pharm. Sci*. **87**: 496–500

Riley, R. J., McGinnity, D. F., Austin, R. P. (2005) A unified model for predicting human hepatic, metabolic clearance from *in vitro* intrinsic clearance data in hepatocytes and microsomes. *Drug Metab. Dispos*. **33**: 1304–1311

Riond, J. L., Riviere, J. E. (1990) Allometric analysis of doxycycline pharmacokinetic parameters. *J. Vet. Pharmacol. Ther*. **13**: 404–407

Ritschel, W. A., Vachharajani, N. N., Johnson, R. D., Hussain, A. S. (1992) The allometric approach for interspecies scaling of pharmacokinetic parameters. *Comp. Biochem. Physiol. C, Comp. Pharmacol. Toxicol*. **103**: 249–253

Roberts, S. A. (2001) High-throughput screening approaches for investigating drug metabolism and pharmacokinetics. *Xenobiotica* **31**: 557–589

Roberts, M. S., Rowland, M. (1985) Hepatic elimination: The dispersion model. *J. Pharm. Sci*. **74**: 585–587

Roberts, M. S., Rowland, M. (1986a) A dispersion model of hepatic elimination: 1. Formulation of the model and bolus considerations. *J. Pharmacokinet. Biopharm*. **14**: 227–261

Roberts, M. S., Rowland, M. (1986b) A dispersion model of hepatic elimination: 2. Steady-state considerations-influence of hepatic blood flow, binding within blood, and hepatocellular enzyme activity. *J. Pharmacokinet. Biopharm*. **14**: 261–288

Roberts, M. S., Rowland, M. (1986c) A dispersion model of hepatic elimination: 3. Application to metabolite formation and elimination kinetics. *J. Pharmacokinet. Biopharm*. **14**: 289–308

Roberts, M. S., Rowland, M. (1986d) Correlation between *in vitro* microsomal enzyme activity and whole organ hepatic elimination kinetics: Analysis with a dispersion model. *J. Pharm. Pharmacol*. **38**: 177–181

Roberts, M. S., Fraser, S., Wagner, A., McLeod, L. (1990) Residence time distributions of solutes in the perfused rat liver using a dispersion model of hepatic elimination: 1. Effect of changes in perfusate flow and albumin concentration on sucrose and taurocholate. *J. Pharmacokinet. Biopharm*. **18**: 209–234

Rowland, M. (1984) Protein binding and drug clearance. *Clin. Pharmacokinet*. **9**(Suppl. 1): 10–17

Rowland, M., Tozer, T. N. (1995) In: Rowland, M., Tozer, T. N. (eds). *Clinical pharmacokinetics: concepts and applications*. Williams & Wilkins, Baltimore, MD

Rowland, M., Benet, L. Z., Graham, G. G. (1973) Clearance concepts in pharmacokinetics. *J. Pharmacokinet. Biopharm*. **1**: 123–136

Rubner, N. (1883) Ueber den einfluss der koepergrosse auf stoff und kraftwechsel. *Z. Biol*. **19**: 535–562

Sahin, S., Rowland, M. (2004) Effect of erythrocytes on the hepatic distribution kinetics of antipyrine. *Eur. J. Drug Metab. Pharmacokinet*. **29**: 37–41

Sanwald-Ducray, P., Dow, J. (1997) Prediction of the pharmacokinetic parameters of reduced dolasteron in man using in vitro-in vivo and interspecies allometric scaling. *Xenobiotica* **27**: 189–201

Saville, B. A., Gray, M. R., Tam, Y. K. (1992a) Models of hepatic drug elimination. *Drug Metab. Rev*. **24**: 49–88

Saville, B. A., Gray, M. R., Tam, Y. K. (1992b) Experimental studies of transient mass transfer and reaction in the liver: Interpretation with a heterogenous compartment model. *J. Pharm. Sci*. **81**: 265–271

Sawada, Y., Hanano, M. Sugiyama, Y., Harashima, H., Iga, T. (1984) Prediction of the volumes of distribution of basic drugs in humans based on data from animals. *J. Pharmacokinet. Biopharm*. **12**: 587–596

Sawada, Y., Hanano, M., Sugiyama, Y., Iga T. (1985) Prediction of the disposition of nine weakly acidic and six weakly basic drugs in humans from pharmacokinetic parameters in rats. *J. Pharmacokinet. Biopharm*. **13**: 477–492

Schmidt-Nielsen, K. (1984) *Why is animal size so important*? Cambridge University Press, Cambridge

Schneider, G., Coassolo, P., Lavé, T. (1999) Combining in vitro and in vivo pharmacokinetic data for prediction of hepatic drug

clearance in humans by artificial neural networks and multivariate statistical techniques. *J. Med. Chem*. **42**: 5072–5076

Schwab, A. J., Barker III, F., Goresky, C. A., Pang, K. S. (1990) Transfer of enalaprilat across rat liver cell membranes is barrier limited. *Am. J. Physiol*. **258**: G461–475

Schwarz, L. R., Schwenk, M., Barth, C., Greim, H. (1979) Studies with isolated liver cells: Effect of taurolithocholate on the transport of taurocholate and bromosulfophtalein. In: Paumgartner, G., Stiel, D. (eds) *Biological effects of bile acids*. MTP Press, Lancaster, pp 127–133

Schwenk, M. (1980) Transport systems of isolated hepatocytes: Studies on the transport of biliary compounds. *Arch. Toxicol*. **44**: 113–126

Shand, D. G., Branch, R. A., Evans, G. H., Nies, A. S., Wilkonson, G. R. (1973) The disposition of propranolol. VII. The effects of saturable hepatic tissue uptake on drug clearance by the perfused rat liver. *Drug Metab. Dispos*. **1**: 679–686

Shibata, Y., Takahashi, H., Ishii, Y. (2000) A convenient in vitro screening method for predicting *in vivo* drug metabolic clearance using isolated hepatocytes suspended in serum. *Drug Metab. Dispos*. **28**: 1518–1523

Shibata, Y., Takahashi, H., Chiba, M., Ishii, Y. (2002) Prediction of hepatic clearance and availability by cryopreserved human hepatocytes: an application of serum incubation method. *Drug Metab. Dispos*. **30**: 892–896

Shim, H. J., Kim, Y. C., Lee, J. H., Kwon, J. W., Kim, W. B., Kim, Y. G., Kim, S. H., Lee, M. G. (2005) Interspecies pharmacokinetic scaling of DA-8159, a new erectogenic, in mice, rats, rabbits and dogs, and prediction of human pharmacokinetics. *Biopharm. Drug Disp*. **26**: 269–277

Siebert, G. A., Hung, D. Y., Chang, P., Roberts, M. S. (2004) Ion-trapping, microsomal binding, and unbound drug distribution in the hepatic retention of basic drugs. *J. Pharmacol. Exp. Ther*. **308**: 228–235

Sietsema, W. K. (1989) The absolute oral bioavailability of selected drugs. *Int. J. Clin. Pharmacol. Ther. Toxicol*. **27**: 179–211

Simeonova, M., Wachner, D., Gimsa, J. (2002) Cellular absorption of electric filed energy: influence of molecular properties of the cytoplasm. *Bioelectrochemistry* **56**: 215–218

Singh, K., Tripp, S. L., Dunton, A. W., Douglas, F. L., Rakhit, A. (1991) Determination of in vivo hepatic extraction ratio from in vitro metabolism by rat hepatocytes. *Drug Metab. Dispos*. **19**: 990–996

Skett, P. (1994) Problems in using isolated and cultured hepatocytes for xenobiotic metabolism/metabolism-based toxicity testing-solutions. *Toxicol. In Vitro* **8**: 491–504

Soars, M. G., Burchell, B., Riley, R. J. (2002) In vitro analysis of human drug glucuronidation and prediction of in vivo metabolic clearance. *J. Pharmacol. Exp. Ther*. **301**: 382–390

Sohlenius-Sternbeck, A.-K., Schmidt, S. (2005) Impaired glutathione-conjugating capacity by cryopreserved human and rat hepatocytes. *Xenobiotica* **35**: 727–736

Steinberg, P., Fischer, T., Kiulies, S., Biefang, K., Platt, K. L., Oesch, F., Bottger, T., Bulitta, C., Kempf, P., Hengstler, J. (1999) Drug metabolizing capacity of cryopreserved human, rat and mouse liver parenchymal cells in suspension. *Drug Metab. Dispos*. **27**: 1415–1422

St-Pierre, M. V., Lee, P. I., Pang, K. S. (1992) A comparative investigation of hepatic clearance models: predictions of metabolite formation and elimination. *J. Pharmacokinet. Biopharm*. **20**: 105–145

Swabb, E. A., Bonner, D. P. (1983) Prediction of aztreonam pharmacokinetics in humans based on data from animals. *J. Pharmacokinet. Biopharm*. **11**: 215–223

Tang, H., Mayersohn, M. (2005) A novel model for prediction of human drug clearance by allometric scaling. *Drug Metab. Dispos*. **33**: 1297–1303

Tang, H., Mayersohn, M. (2006a) On the observed large interspecies overprediction of human clearance ("vertical allometry") of UCN-01: Further support for a proposed model based on plasma protein binding. *J. Clin. Pharmacol*. **46**: 398–400

Tang, H., Mayersohn, M. (2006b) A global examination of allometric scaling for predicting human drug clearance and the prediction of large vertical allometry. *J. Pharm. Sci*. **95**: 1783–1799

Terelius, Y., Hagbjörk, A. L., Stjernström, H., Gabrielsson, J. (2001) AR-A000002, a high affinity 5-HT_{1B} receptor antagonist: pharmacokinetic scaling studies. *Eur. Neuropsychopharmacol*. **3**: 213

Thummel, K. E., Kunze, K. L., Shen, D. D. (1997) Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv. Drug Del. Rev*. **27**: 99–127

Travis, C. C., White, R. K., Ward, R. C. (1990) Interspecies extrapolation of pharmacokinetics. *J. Theor. Biol*. **142**: 285–304

Treijtel, N., Barendregt, A., Freidig, A. P., Blaauboer, B. J., van Eijkeren, J. C. H. (2004) Modeling the in vitro intrinsic clearance of the slowly metabolized compound tolbutaminde determined in sandwich-cultured rat hepatocytes. *Drug Metab. Dispos*. **32**: 884–891

Tucker, G. T., Houston, J. B., Huang, S.-M. (2001) Optimizing drug development: Strategies to assess drug metabolism/transporter interaction potential: toward a consensus. *Pharm. Res*. **18**: 1071–1080

Ubeaud, G., Schmitt, C., Jaeck, D., Lavé, T., Coassolo, P. (1995) Bosentan, a new endothelin receptor antagonist: prediction of the systemic plasma clearance in man from combined in vivo and in vitro data. *Xenobiotica* **25**: 1381–1390

van de Waterbeemd, H., Gifford, E. (2003) ADMET in silico modeling: Towards prediction paradise? *Nature Rev. Drug Disc*. **2**: 192–204

van Eijkeren, J. C. (2002) Estimation of metabolic rate constants in PBPK-models from liver slice experiments: What are the experimental needs? *Risk Anal*. **22**: 159–173

van Hoogdalem, E. J., Soeishi, Y., Matsushima, H., Higuchi S. (1997) Disposition of the selective alpha1A-adrenoceptor antagonist tamsulosin in humans: comparison with data from interspecies scaling. *J. Pharm. Sci*. **86**: 1156–1161

Videla, L., Flattery, K. V., Sellers, E. A., Israel, Y. (1975) Ethanol metabolism and liver oxidative capacity in cold acclimation. *J. Pharmacol. Exp. Ther*. **192**: 575–582

von Richter, O., Greiner, B., Fromm, M. F., Fraser, R., Omari, T., Barclay, M. L., Dent, J., Somogyi, A. A., Eichelbaum, M. (2001) Determination of in vivo absorption, metabolism, and transport of drugs by the human intestinal wall and liver with a novel perfusion technique. *Clin. Pharmacol. Ther*. **70**: 217–227

Wajima, T., Fukumura, K., Yano, Y., Oguma, T. (2002) Prediction of human clearance from animal data and molecular structural parameters using multivariate regression analysis. *J. Pharm. Sci*. **91**: 2489–2499

Wajima, T., Fukumura, K., Yano, Y., Oguma, T. (2003) Prediction of human pharmacokinetics from animal data and molecular structural parameters using multivariate regression analysis: Oral clearance. *J. Pharm. Sci*. **92**: 2427–2440

Wajima, T., Yano, Y., Fukumura, K., Oguma, T. (2004) Prediction of human pharmacokinetic profile in animal scale up based on normalizing time course profiles. *J. Pharm. Sci*. **93**: 1890–1900

Walker, C. H. (1978) Species differences in microsomal monooxygenase activity and the relationship of biological half-lives. *Drug Met. Rev*. **7**: 295–323

Ward, K. W., Smith, B. R. (2004) A comprehensive quantitative and qualitative evaluation of extrapolation of intravenous pharmacokinetic parameters from rat, dog, and monkey to humans. I. Clearance. *Drug Metab. Dispos*. **32**: 603–611

Ward, K. W., Azzarano, L. M., Bondinell, W. E., Cousins, R. D., Huffman, W. F., Lakas, D. R., Keenan, R. M., Ku, T. W.,

Lundberg, D., Miller, W. H., Mumaw, J. A., Newlander, K. A., Pirhalla, J. L., Roethke, T. J., Salyers, K. L., Souder, P. R., Stelman, G. J., Smith, B. J. (1999) Preclinical pharmacokinetics and interspecies scaling of a novel vitronectin receptor antagonist. *Drug Metab. Dispos*. **27**: 1232–1241

Ward, K. W., Proksch, J. W., Gorycki, P. D., Yu, C.-P., Ho, M. Y. K., Bush, B. D., Levy, M. A., Smith, B. R. (2002) SB-242235, a selective inhibitor of p38 mitogen-activated protein kinase. II: *in vitro* and *in vivo* metabolism studies and pharmacokinetic extrapolation to man. *Xenobiotica* **32**: 235–250

Weibel, E. R., Staubli, W., Gnagi, H. R., Hess, F. A. (1969) Correlated morphometric and biochemical studies on the liver cell. Morphometric model, and normal morphometric data for rat liver. *J. Cell Biol*. **42**: 68–91

Weisiger, R. A. (1985) Dissociation from albumin: A potentially rate-limiting step in the clearance of substrates by the liver. *Proc. Natl. Acad. Sci. USA* **82**: 1563–1567

Weisiger, R. A., Pond, S., Bass, L. (1991) Hepatic uptake of protein-bound ligands: Extended sinusoidal perfusion model. *Am. J. Physiol*. **261**: G872–884

Weiss, M., Sziegoleit, W., Forster, W. (1977) Dependence of pharmacokinetic parameters on the body weight. *Int. J. Clin. Pharmacol. Biopharm*. **15**: 572–575

Weiss, T. S., Jahn, B., Cetto, M., Jauch, K.-W., Thasler, W. E. (2002) Collagen sandwich culture affects intracellular polyamine levels of human hepatocytes. *Cell Prolif*. **35**: 257–267

Wessel, M. D., Jurs, P. C., Tolan, J. W., Muskal, S. M. (1998) Prediction of human intestinal absorption of drug compounds from molecular structure. *J. Chem. Inf. Comp. Sci*. **38**: 726–735

West, G. B., Brown, J. H., Enquist, B. J. (1997) A general model for the origin of allometric scaling laws in biology. *Science* **276**: 122–126

Wilkinson, G. R., Shand, D. G. (1975) Commentary – A physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther*. **18**: 377–390

Winkler, K., Bass, L., Keiding, S., Tygstrup, N. (1974) The effect of hepatic perfusion on the assessment of kinetic constants in regulation of hepatic metabolism. In: Lundquist, F., Tygstrup, N. (eds) *A Benzon Symposium VI*. Munksgaard, Copenhagen, pp 797–807

Worboys, P. D., Bradbury, A., Houston, J. B. (1995) Kinetics of drug metabolism in rat liver slices. Rates of oxidation of ethoxycoumarin and tolbutamide, examples of high- and low-clearance compounds. *Drug Metab. Dispos*. **23**: 393–397

Worboys, P. D., Bradbury, A., Houston, J. B. (1996a) Kinetics of drug metabolism in rat liver slices. II. Comparison of clearance by liver slices and freshly isolated hepatocytes. *Drug Metab. Dispos*. **24**: 676–681

Worboys, P. D., Bradbury, A., Houston, J. B. (1996b) Kinetics of drug metabolism in rat liver slices. III. Relations between metabolic clearance and slice uptake rate. *Drug Metab. Dispos*. **24**: 460–467

Worboys, P. D., Brennan, B., Bradbury, A., Houston, J. B. (1997) Metabolic kinetics of ondansetron in rat. Comparisons of hepatic microsomes, isolated hepatocytes and liver slices with in vivo disposition. *Xenobiotica* **26**: 897–907

Wortelboer, H. M., De Kruif, C. A., van Iersel, A. A. J, Falke, H. E., Noordhoek, J., Blaauboer, B. J. (1990) The isoenzyme pattern of cytochrome P450 in rat hepatocytes in primary culture, comparing different enzyme activities in microsomal incubations and in intact monolayers. *Biochem. Pharmacol*. **40**: 2525–2534

Xu, X., Schwab, A. J., Barker III, F., Goresky, C. A., Pang, K. S. (1994) Salicylamide sulphate cell entry in perfused rat liver: A multiple-indicator dilution study. *Hepatology* **19**: 229–244

Yamasaki, S., Toth, L. N., Black, M. L., Duncan, J. N. (2004) Comparison of prediction methods for in vivo clearance of (S, S)-3-[3-(methylsulfonyl)phenyl]-1-propylpiperidine hydrochloride, a dopamine D2 receptor antagonist, in humans. *Drug Metab. Dispos*. **32**: 398–404

Yates, F. E., Kugler, P. N. (1986) Similarity principles and intrinsic geometries: Contrasting approaches to interspecies scaling. *J. Pharm. Sci*. **75**: 1019–1027

Zomorodi, K., Carlile, D.J., Houston, J. B. (1995) Kinetics of diazepam metabolism in rat hepatic microsomes and hepatocytes and their use in predicting *in vivo* hepatic clearance. *Xenobiotica* **25**: 907–916

Zuegge, J., Schneider, G., Coassolo, P., Lavé, T. (2001) Prediction of hepatic metabolic clearance. *Clin. Pharmacokinet*. **40**: 553–563