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An Allometric Model for Predicting Blood Ethanol Elimination in Mammals

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ABSTRACT. The relationship of ethanol elimination kinetics in mammals was estimated using the allometric principle. The hypothesis of relationships between parameters obtained from the compartment model with Michaelis–Menten elimination kinetics and body weight can lead to common equations of blood ethanol elimination in mammals. The maximum elimination velocity (g/hr) and the apparent volume of distribution (L) were significantly proportional to the 0.71 and 0.93 powers of body weight ($r = 0.994$, $P < 0.01$ and $r = 0.998$, $P < 0.001$), respectively. There was no significant relationship between the Michaelis constant and body weight. In the differential equations of the two-compartment model, the kinetics parameters were substituted for the obtained power functions. Good fitting of these equations for the real data showed that ethanol elimination kinetics in mammals can be predicted quantitatively. BIOCHEM PHARMACOL **57**;2:219–223, 1999. © 1998 Elsevier Science Inc.

KEY WORDS. ethanol; pharmacokinetics; elimination; scaling; allometry; mammal

Numerous researchers have studied EtOH† metabolism in various species, i.e. mice, rats, rabbits, guinea pigs, dogs, sheep, horses, and humans. Blood EtOH is eliminated mainly via the liver, regardless of the species. Hepatic ADH plays a major role in hepatic metabolism [1]. Other enzyme pathways such as the microsomal EtOH oxidizing system (MEOS) and catalase also contribute to the metabolism [1]. Thus, there is no large difference in the EtOH elimination pathway among species. However, there is an obvious interspecies variation in the elimination rate of EtOH [2, 3]. Lester and Keokosky [4] reported a similarity in alcohol oxidation rates among rats, horses, and humans as the first example of pharmacokinetic scaling. They revealed that the apparent zero-order oxidation rate varied only from 0.24 to 0.39 g/kg$^{0.75}$/hr. Vestal *et al.* [5] extended this analysis, demonstrating a linear relationship between the rate of EtOH metabolism (mg/kg/hr) and metabolic rate (L O_2/kg/day) in twelve species ($r = 0.95$, $P < 0.001$). However, Boxenbaum [6] indicated that these scaling reports did not consider that the EtOH elimination rate varies dose dependently by using the zero-order model. Since the 1980s, it has been well-known that EtOH elimination follows Michaelis–Menten kinetics. No scaling study with the Michaelis–Menten model has been reported.

In the Michaelis–Menten model, EtOH elimination kinetics can be assessed by the elimination rate, consisting of the elimination velocity and the volume of distribution, and the Michaelis constant, K_m. The elimination velocity is a physiological parameter, whereas the volume of distribution is a constitutional one. If there are interspecies relationships in these parameters, we can estimate the EtOH elimination kinetics quantitatively in various species. As regards the physiological parameter, it is empirically known that physiological time, such as circulation time or respiratory interval, is proportional to the 0.25 power of body weight [7]. Metabolic rate is related to body weight by a power of 0.75 [8]. With regard to the constitutional parameter, internal organ weight is proportional to a power of body weight. Such a power equation of body weight (W), the form of which is $Y = aW^x$, is called the allometric equation [6]. The pharmacokinetic extrapolation of human kinetics from the data of animals, using the allometric equation, has been reported [9–11].

Our hypothesis states that interspecies variation in EtOH elimination may be caused by interspecies variation in the elimination rate and/or the volume of distribution. If these parameters are proportional to a power of body weight, there may be an interspecies relationship in ethanol elimination. If our hypothesis is correct, the differential equations of the two-compartment model can be represented as functions of body weight. Therefore, the objectives of the present study were: (1) to estimate the relationship between kinetics parameters and body weight obtained from references, and (2) to evaluate the possibility of deriving common equations of blood EtOH elimination in mammals by using these relationships.

MATERIALS AND METHODS

Allometric Analysis

Each physiological, biophysical, or biochemical variable in animals can be represented as a power equation of body weight, i.e. the allometric equation [9–11]:

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† *Abbreviations:* ADH, alcohol dehydrogenase; EtOH, ethanol; and 2CAM, the two-compartment allometric model with Michaelis–Menten elimination kinetics.

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$$Y = aW^b \quad (1)$$

where Y is a physiological or anatomical variable, W is body weight (kg), a is the allometric constant, and b is the allometric exponent. This equation can be represented by the logarithmic transformation:

$$\log Y = \log a + b \log W \quad (2)$$

Therefore, Y is proportional to W on a logarithmic scale.

We obtained the kinetic parameters for the compartment Michaelis–Menten model in mammals from the published literature (Table 1). The relationship between each parameter and body weight (Eq. 2) was estimated by linear regression analysis of logarithmic data without the weighting function [19]. The significance of the relationship was tested using the correlation coefficient and P value.

Simulation of EtOH Elimination Kinetics with the Two-compartment Michaelis–Menten Model

The two-compartment model with Michaelis–Menten elimination kinetics follows:

$$-\frac{\partial C_1(t)}{\partial t} = \left[\frac{V_{max}}{K_m + C_1(t)} + K_{12}\right] \cdot C_1(t) - K_{21} \cdot \frac{V_2}{V_1} \cdot C_2(t),\ C_1(0) = D/V_1 \quad (3)$$

$$-\frac{\partial C_2(t)}{\partial t} = -K_{12} \cdot \frac{V_1}{V_2} \cdot C_1(t) + K_{21} \cdot C_2(t),\quad C_2(0) = 0 \quad (4)$$

where V_{max} is the apparent maximum elimination velocity (mg/mL/hr); K_m is the Michaelis constant (mg/mL); $C(t)$ is the EtOH plasma concentration at time t; and $C_1(t)$ and $C_2(t)$ are the EtOH concentrations (mg/mL) in central and peripheral compartments, respectively. V_1 and V_2 are the volumes of distribution (L/kg) in central and peripheral compartments, respectively; K_{12} and K_{21} are the first-order partition rate constants (hr^{-1}). There is a relationship of $K_{12}/K_{21} = V_2/V_1$. V_d, the apparent steady-state volume of distribution (L/kg), is the product of V_1 and V_2. Therefore, the EtOH concentration–time courses in ethanol elimination are reconstituted from Eqs. 3 and 4 as follows:

$$-\frac{\partial C_1(t)}{\partial t} = \left[\frac{V_m/V_d}{K_m + C_1(t)} + \frac{CL_{12}}{V_1}\right] \cdot C_1(t) - \frac{CL_{21}}{V_1} \cdot C_2(t),\ C_1(0) = \frac{D \cdot W}{V_1} \quad (5)$$

$$-\frac{\partial C_2(t)}{\partial t} = -\frac{CL_{12}}{V_d - V_1} \cdot C_1(t) + \frac{CL_{21}}{V_d - V_1} \cdot C_2(t),\quad C_2(0) = 0 \quad (6)$$

TABLE 1. Mean pharmacokinetic parameters in one- and two-compartment models with Michaelis–Menten elimination kinetics reported for mammals

Species	N	W (kg)	Compartment number	V_{max} (mg/mL/hr)	K_m (mg/mL)	V_1 (L/kg)	V_d* (L/kg)	K_{12} (hr^{-1})	K_{21} (hr^{-1})	V_d (L)	V_m (g/hr)	CL_{12} (L/hr)	CL_{21} (L/hr)	References
Rat	16	0.256 ± 0.090	2	0.70 ± 0.03	0.07 ± 0.01	0.66 ± 0.02	0.78	0.15 ± 0.06	0.80 ± 0.03	0.200	0.140	0.0253	0.0246	[12]
Rat†	4	0.240 ± 0.006	2	1.080 ± 0.012	0.118 ± 0.038	0.407 ± 0.021	0.785	18.9 ± 4.80	20.3 ± 2.4	0.188	2.03	1.85	1.85	[13]
Rabbit	20	2.20	2	0.56 ± 0.04	0.12 ± 0.02	0.40 ± 0.02	0.76	1.60 ± 0.38	1.80 ± 0.30	1.67	0.936	1.41	1.43	[14]
Macaque	6	4.65 ± 0.50	1	0.261 ± 0.050	0.05 ± 0.015		0.83 ± 0.05			3.86 ± 0.46	1.01 ± 0.26			[15]
Sheep‡	3	48.3 ± 7.3	1	0.292 ± 0.015	0.319 ± 0.024		0.675‡			32.6	8.19			[16]
Human‡	8	78.0	2	0.255	0.03		0.47			36.66	9.36			[17]
Human	6	80.1 ± 5.1	1	0.232 ± 0.026	0.821 ± 0.0287		0.535 ± 0.050			42.7 ± 3.3	9.91			[18]

Values are means ± SD except where noted.
*V_d [L/kg] = $V_1 \cdot (K_{12} + K_{21})/K_{21}$.
†Mean ± SEM.
‡Values are median.

where V_m is the apparent maximum elimination rate (g/hr), and CL_{12} and CL_{21} are the clearances between central and peripheral compartments (L/hr) and equal to $K_{12} \cdot V_1$ and $K_{21} \cdot V_2$. The substitution of power equations obtained for the kinetic parameters in Eqs. 5 and 6 brought the differential equations to functions of body weight (W) and dosage (D). Equations for simulation were obtained by substituting (0.5, 0.245), (1, 0.2599), (2, 0.2685), (3, 0.2386), and (0.75, 78) for (D, W) in the differential equations, expressed as functions of body weight and dosage. The simulation data were calculated from these simulated equations by the Runge-Kutta-Gill method using MULTI(RUNGE) [20] on the 64-bit computer, DEC Multia. The bias and the precision of the model were measured by calculating mean prediction error (*MPE*) and the root mean square prediction error (*RMSE*) according to the following equations [21]:

$$MPE = \frac{\Sigma(\text{predicted} - \text{observed})}{n} \quad (7)$$

$$MSE = \frac{\Sigma(\text{predicted} - \text{observed})^2}{n} \quad (8)$$

$$RMSE = \sqrt{MSE} \quad (9)$$

Statistics

The experimental data are presented as means ± SD. Mean values were compared according to one-way ANOVA with Duncan's multiple range test and Student's *t*-test. Differences with a $P < 0.05$ were considered significant. In the regression analysis, significance was tested using *P* values of the correlation coefficient and parameters for the regression line.

RESULTS

Allometric Approach to Parameters

Figures 1 and 2 show the relationships between V_d, V_m, and body weight, respectively. V_{max}, that is, the quotient of V_m and V_d, was represented as $0.591W^{0.224}$. For example, in a man of 70 kg body weight, V_d and V_{max} were 39.9 (L) and 0.228 (mg/mL/hr), respectively, which were similar to the values reported by Wilkinson *et al.* [18]. There was no significant relation between K_m and body weight, and the mean of K_m was 0.085 (mg/mL).

Table 2 shows parameters of allometric equations, correlation coefficients, and *P* values from data in the two-compartment model. From the *P* values of the parameters and the correlation coefficients, we determined that there were significant relationships between V_m, V_d, V_1, and body weight. The clearances between central and peripheral compartments, CL_{12} and CL_{21}, were not significantly correlated to body weight on the logarithmic coordinates, the means ± SD of which were 1.09 ± 0.95 (L/hr) and 1.10 ± 0.95 (L/hr), respectively.

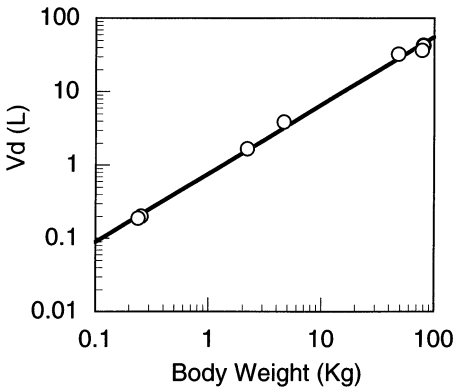

FIG. 1. Interspecies relationship between the apparent volume of distribution (V_d) and body weight in the Michaelis–Menten model. The regression line is $V_d = 0.762W^{0.932}$ ($r = 0.9984$, $P < 0.001$).

Simulation of EtOH Elimination Kinetics

Substituting allometric equations (Table 2) and the constant values described above for the parameters in Eqs. 5 and 6, the definitive equations of EtOH concentration in 2CAM are approximated as follows:

$$-\frac{\partial C_1(t)}{\partial t} = 2.5\left[\left(\frac{0.24W^{0.66}}{0.085 + C_1(t)} + 1\right)C_1(t) - C_2(t)\right]W^{-0.89},\ C_1(0) = 2.3DW^{0.11} \quad (10)$$

$$-\frac{\partial C_2(t)}{\partial t} = -\frac{1.1}{0.76W^{0.93} - 0.44W^{0.89}} \cdot [C_1(t) - C_2(t)],\ C_2(0) = 0 \quad (11)$$

We carried out a simulation to evaluate the validity of 2CAM. Figure 3 shows the simulation curves of 2CAM when D is 0.5, 1, 2, or 3 (g/kg) and W is 245.0, 259.9, 268.5, or 238.6 (g); the mean EtOH concentration–time

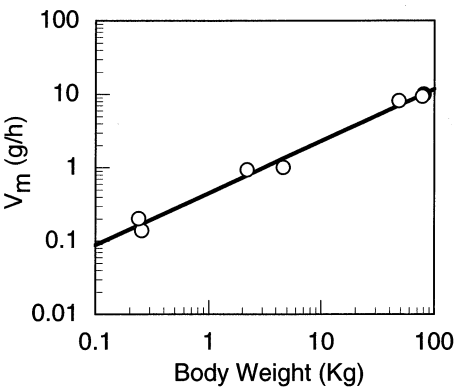

FIG. 2. Interspecies relationship between the apparent maximum EtOH elimination rate (V_m) and body weight. The straight line is the regression line of $V_m = 0.450W^{0.708}$ ($r = 0.9944$, $P < 0.01$).

TABLE 2. Interspecies parameters of the allometric relationship: log Y = log a + b log W in the two-compartment Michaelis–Menten model

Kinetic parameter (y)*	a	b	Correlation coefficient (r)	P value
V_m (g/hr)	0.450	0.708	0.9944	<0.01
V_d (L)	0.762	0.932	0.9984	<0.001
V_1 (L)	0.441	0.886	0.9767	<0.02
CL_{12} (L/hr)	0.695	0.814	0.4274	>0.05
CL_{21} (L/hr)	0.696	0.827	0.4306	>0.05

*Y is a parameter obtained by using the two-compartment model as shown in Table 1.

courses in rats were obtained from our previous report [12]. We obtained *MPE* and *RMSE* values of 0.0393 and 0.2695, respectively. *MPE* is a convenient measure of bias, and *RMSE* is one of prediction. Therefore, the time–course data obtained from 2CAM were well predicted for the rat real-time data. This finding shows that 2CAM can be useful for quantitatively predicting blood EtOH elimination in mammals.

DISCUSSION

We assessed interspecies relationships of kinetic parameters by linear regression analysis using logarithmic transformation of the allometric equation. The magnitudes of the random errors in such parameters are independent of body weight. However, transformation of the data into linear form by taking logarithms will obviously produce data in which the errors in log Y are not independent of log W [19]. In the present study, therefore, no weighting function was used for linear regression analysis by logarithmic transformation of the allometric equation. Recently, Ebert and Russell [22] reported a new model of allometry. They suggest that nonlinear regression analysis should be used for allometric data, and the allometric equation composed of error term should be applied. However, it is difficult to apply that approach for a large variation of body weights and a small data set, as in the present study.

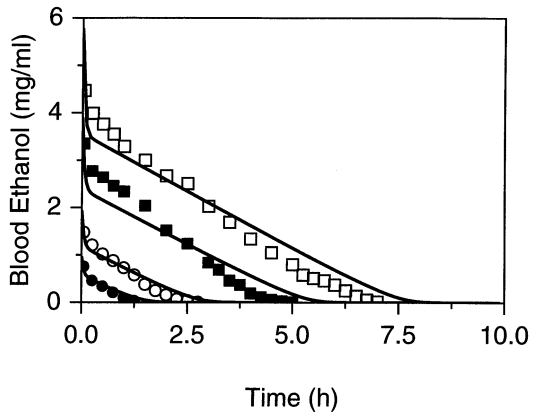

FIG. 3. Comparison between the simulation of EtOH elimination and rat published data. The plots are mean EtOH concentrations after the doses of 0.5 (●), 1 (○), 2 (■), and 3 (□) g/kg from Ref. 12. The lines are simulation curves by 2CAM (the two-compartment allometric model, Eqs. 10 and 11) substituting 0.5 (g/kg) and 0.245 (kg), 1 and 0.2599, 2 and 0.2685, and 3 and 0.2386, respectively, as published data sets.

Adolph [9] observed that many anatomical and physiological variables can be correlated among mammals as exponential functions of body weight. Dedrick [10] and Boxenbaum [6, 11] developed the interspecies scaling in pharmacokinetic fields. They applied it to linear pharmacokinetic parameters such as volume of distribution and total clearance. In EtOH elimination, volume of distribution, V_d, is not varied, but total clearance is changed dose-dependently for the nonlinearity. However, the maximum elimination rate, V_m, and the Michaelis constant, K_m, as components of total clearance, are constant values in each animal. Clearances (CL_{12} and CL_{21}) between compartments in the two-compartment model are also constant in each animal. Since V_m indicates a physiological parameter, it can be scaled-up. Taberner [23] indicated that it is important to be aware of the many pitfalls inherent in fitting a straight line to a single point and translating the scientific probability into the legal requirement of "beyond reasonable doubt". Wilkinson [24] also reported that in many instances the linear correlation coefficient associated with the log-log allometric plots can be greater than 0.90, which indicates an acceptable level of prediction. Therefore, the coefficient of V_d and V_1 obtained from the present study, which are constitutional parameters, would be acceptable. The correlation coefficients between the maximum elimination rate V_m (g/hr) and body weight should also be sufficient for prediction. These findings support our hypothesis that pharmacokinetic parameters in the nonlinear kinetics can be scaled-up among species and treated as well as the parameters in linear kinetics.

The volume of distribution, V_d, was proportional to the 0.932 power of body weight (Fig. 1). This exponent value is similar to the value reported by Boxenbaum [6]. A constant value of 0.762 in this allometric equation is close to that of antipyrine [6], but not close to those of methotrexate [6] and cyclophosphamide [6]. Antipyrine is well known to follow non-linear kinetics under a common dose. It has been reported that V_d indicates the total body water volume [25]. The relationship between V_d and body weight, $V_d = 0.762W^{0.932}$, shows an interspecies relationship in total body water volume. In general, a constitutional parameter is proportional to body weight. The allometric exponent of 0.932 for V_d was within the typical range for

the exponent (between 0.8 and 1.0) [26]. The metabolic rate is theoretically proportional to the 0.75 power of body weight [6–8, 11]. Our exponent of 0.708 for V_m certainly falls within the range of values that have been reported for metabolic rate, i.e. 0.66 to 0.77 [26]. These findings suggest the precision of our allometric analysis of ethanol elimination kinetics. The absence of a significant difference of K_m among species suggests that there is no large interspecies variation in the value after intravenous administration. It is well known that there exists a polymorphism of metabolic enzymes, e.g. ADH [1], aldehyde dehydrogenase [1], and cytochrome P450 2E1 [27], in various species. Many researchers have studied the effects of the polymorphism on EtOH kinetics in each species. However, the differences of the effects among species have never been studied. The use of the 2CAM equations for such a case may play an important role in the analysis of these differences, and someone may find the biological significance of the polymorphism of the alcohol-metabolizing enzymes. The 2CAM equation obtained from the present study holds under the conditions of male, fasted state, and intravenous administration of EtOH. However, the effects of interspecies differences, such as gender, nutrition, and pathophysiology, on the 2CAM equation need to be clarified, and then the 2CAM equation can be applied for various conditions so that it will be a very useful tool for alcohol research.

We computed allometric equations of the two-compartment Michaelis–Menten model (2CAM). The good fit of 2CAM-simulated curves to the published blood data sets proves that EtOH elimination in various species can be predicted quantitatively. 2CAM equations are functions of body weight and dosage. Therefore, if body weight and intravenous dosage in a species are known, we can predict the EtOH elimination time course of the animal.

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References

1. Lieber CS, Biochemical and molecular basis of alcohol-induced injury to liver and other tissues. *New Engl J Med* **319:** 1639–1650, 1988.
2. Coldwell BB and Platonow N, The effect of methylmercuric acetate on the rate of disappearance of ethanol from the blood of swine. *Toxicol Appl Pharmacol* **14:** 368–375, 1969.
3. Newman HW and Lehman AJ, Rate of disappearance of alcohol from the blood stream in various species. *Arch Int Pharmacodyn Ther* **55:** 440–446, 1937.
4. Lester D and Keokosky WZ, Alcohol metabolism in the horse. *Life Sci* **6:** 2313–2319, 1967.
5. Vestal RE, McGuire EA, Tobin JD, Andres R, Norris AH and Mezey E, Aging and ethanol metabolism. *Clin Pharmacol Ther* **21:** 343–354, 1977.
6. Boxenbaum H, Interspecies pharmacokinetic scaling and the evolutionary-comparative paradigm. *Drug Metab Rev* **15:** 1071–1121, 1984.
7. Kleiber M, Metabolic turnover rate: A physiological meaning of the metabolic rate per unit body weight. *J Theor Biol* **53:** 199–204, 1975.
8. Prothero JW, Maximal oxygen consumption in various animals and plants. *Comp Biochem Physiol* **64A:** 463–466, 1979.
9. Adolph EF, Quantitative relations in the physiological constitutions of mammals. *Science* **109:** 579–585, 1949.
10. Dedrick RL, Animal scale-up. *J Pharmacokinet Biopharm* **1:** 435–461, 1973.
11. Boxenbaum H, Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. *J Pharmacokinet Biopharm* **10:** 201–227, 1982.
12. Matsumoto H, Fujimiya T and Fukui Y, Role of alcohol dehydrogenase in rat ethanol elimination kinetics. *Alcohol Alcohol* **29:** 15–20, 1994.
13. Shimada J, Miyahara T, Otsubo S, Yoshimatsu N, Oguma T and Matsubara T, Effects of alcohol-metabolizing enzyme inhibitors and β-lactam antibiotics on ethanol elimination in rats. *Jpn J Pharmacol* **45:** 533–544, 1987.
14. Fujimiya T, Yamaoka K and Fukui Y, Parallel first-order and Michaelis–Menten elimination kinetics of ethanol. Respective role of alcohol dehydrogenase (ADH), non-ADH and first-order pathways. *J Pharmacol Exp Ther* **249:** 311–317, 1989.
15. Kalhorn TF, Bowden DM and Slattery JT, Pharmacokinetics of ethanol in pigtailed macaques: Intersubject variability and effect of subchronic administration. *Pharmacol Biochem Behav* **24:** 485–489, 1986.
16. Jean-Blain C, Durix A and Tranchant B, Kinetics of ethanol metabolism in sheep. *Reprod Nutr Dev* **32:** 83–90, 1992.
17. Rangno RE, Kreeft JH and Sitar DS, Ethanol 'dose-dependent' elimination: Michaelis–Menten v classical kinetic analysis. *Br J Clin Pharmacol* **12:** 667–673, 1981.
18. Wilkinson PK, Sedman AJ, Sakmar E, Earhart RH, Weidler DJ and Wagner JG, Blood ethanol concentrations during and following constant-rate intravenous infusion of alcohol. *Clin Pharmacol Ther* **19:** 213–223, 1976.
19. Miller JC and Miller JN, *Statistics for Analytical Chemistry*, 3rd Edn, pp. 124–141. Ellis Horwood Limited, Chichester, U.K., 1993.
20. Yamaoka K and Nakagawa T, A nonlinear least squares program based on differential equations, MULTI(RUNGE), for microcomputers. *J Pharmacobio-Dyn* **6:** 595–606, 1983.
21. Sheiner LB and Beal SL, Some suggestions for measuring predictive performance. *J Pharmacokinet Biopharm* **9:** 503–512, 1981.
22. Ebert TA and Russell MP, Allometry and model II non-linear regression. *J Theor Biol* **168:** 367–372, 1994.
23. Taberner PV, Pharmacokinetics of alcohol and the law. *Trends Pharmacol Sci* **9:** 47–48, 1988.
24. Wilkinson GR, Clearance approaches in pharmacology. *Pharmacol Rev* **39:** 1–47, 1987.
25. Jones AW, Hahn RG and Stalberg HP, Pharmacokinetics of ethanol in plasma and whole blood: Estimation of total body water by the dilution principle. *Eur J Clin Pharmacol* **42:** 445–448, 1992.
26. Mordenti J and Chapell W, The use of interspecies scaling in toxicokinetics. In: *Toxicokinetics and New Drug Development* (Eds. Yacobi A, Skelly J and Bartra V), pp. 42–96. Pergamon Press, New York, 1989.
27. Lieber CS, Cytochrome P450 2E1: Its physiological and pathological role. *Physiol Rev* **77:** 517–544, 1997.