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Residence Time Distribution of Diazepam in the Isolated Perfused Rat Liver. Analysis with the Axial Dispersion Model

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The application of the axial dispersion model to diazepam hepatic elimination was evaluated using data obtained for impulse-response experiments with diazepam in the single-pass isolated perfused rat liver preparation. The transient form of the two-compartment dispersion model was applied to the output concentration versus time profile of diazepam after bolus input of a radiolabelled tracer into the hepatic portal vein (n = 4), providing D_N and CL_{int} estimates of 0.251 ± 0.093 and 135 ± 59 ml min^{-1}, respectively. In contrast, the one-compartment form of the axial dispersion model, which assumes instantaneous transversal distribution of substance to the accessible spaces within the liver, could not adequately describe the residence time distribution (RTD) of diazepam. Furthermore, the magnitude of D_N, a stochastic parameter which characterizes the axial spreading of solutes during transit through the liver, is similar to that determined for non-eliminated substances such as erythrocytes, albumin, sucrose and water. These findings suggest that the dispersion of diazepam in the perfused rat liver is determined primarily by the architecture of the hepatic microvasculature.

Key words: Diazepam, Hepatic elimination, Physiologic models, Dispersion model, Isolated Perfused rat liver.

The fraction of drug escaping extraction during a single passage through the liver (availability) depends on the fraction of drug unbound in blood, organ perfusion, hepatocellular enzyme activity, and, in some cases, the permeability of the hepatocyte membrane of the drug. Various models have been used to describe the in-

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fluence of these physiological determinants on hepatic drug clearance and availability (1). The fundamental difference between these models lies in the assumption applied to the extent of axial spreading of substrate within the hepatic microvasculatory system. At the two extremes are the *venous equilibrium model* (well-stirred model) and the *undistributed parallel-tube model* (9, 20).

Because neither of these simplistic models is entirely compatible with the growing body of experimental data on the hepatic handling of drugs, and with the known morphological features of the liver (14), models which apply a more realistic view of hepatic microvasculatory processes, such as the *distributed parallel-tube models* (2, 7, 17) and the *axial dispersion model* (13) may be more appropriate. These more complex models attempt to account for the known heterogeneity of the microvasculature, and in some cases the enzyme activity, of the liver. In the distributed parallel-tube models, the degree of heterogeneity is dictated by the variance of the statistical distribution chosen to represent the particular property. In the axial dispersion model, organ blood flow heterogeneity is reflected by the magnitude of the dispersion number, D_N. The undistributed parallel-tube model and the venous equilibrium model are extreme forms of the axial dispersion model in which D_N approaches 0 or infinity, respectively (14). The dispersion model has also been modified to incorporate transverse heterogeneity of enzyme activity (3).

The axial dispersion model has been proved to successfully predict the hepatic disposition of a number of non-eliminated substances (14). Nevertheless, it has not been extensively applied to substances for which hepatic extraction may influence the shape of the residence time distribution. The primary objective of this communication, therefore, is to test the applicability of the axial dispersion model of drug elimination to the hepatic RTD of diazepam and the isolated perfused rat liver preparation.

Materials and Methods

Unlabelled diazepam and 2-[^{14}C]-diazepam (purity 99 % TLC, 194 μCi/mg) were obtained from Roche (Basel, Switzerland) and human serum albumin (HSA) was obtained from Kabi AB (Sweden). HPLC solvents were purchased from British Drug Houses. All other reagents were of analytical grade.

Protein binding determination. — The binding of diazepam within perfusates containing varying concentrations of HSA was determined by equilibrium dialysis (Dianorm®, Switzerland) with Spectra/Por-2 membranes (Spectrum Medical Industries Inc., California, U.S.A.). Briefly, 1 ml of perfusate containing 1 mg l^{-1} of diazepam and 0.005 μCi of ^{14}C-diazepam were dialysed against 1 ml of protein-free perfusate at 37 °C for 4 h. ^{14}C-diazepam was determined in a 0.2 ml aliquot of each compartment by radiochemical analysis (LKB Rackbeta liquid scintillation counter, Finland). The unbound fraction of diazepam was taken to be the ratio of the concentration of radiolabelled diazepam in the protein-free buffer, to that in the perfusate compartment, at the end of dialysis.

Diazepam HPLC assay. — Diazepam was assayed by HPLC using a modification of the method of RAISYS *et al.* (11). All glassware was silanized. Briefly, 50 μl of internal standard solution (nitrazepam, 3.75 μg ml^{-1}; Sigma) and 200 μl of acetonitrile (to precipitate HSA) were added to 200 μl of sample. After vortex mixing (1 min) and centrifugation (3400 rpm, 20 min) 20 μl of the supernatant was injected into the HPLC system. Calibration curves were constructed over the range of 100 to 1500 ng ml^{-1} and the intraday coefficient

of variation (n = 5) ranged between 7.42 % (100 ng ml^{-1}) and 3.54 % (1500 ng ml^{-1}).

Hepatic outflow samples obtained during perfusion with protein-free perfusate contained concentrations of diazepam that could not be measured accurately using the method described above. Therefore it was necessary to perform a simple extraction procedure prior to HPLC analysis of such samples. Briefly, 50 µl of internal standard solution (nitrazepam, 3.75 µg ml^{-1}) and 8 ml of hexane/ethylacetate (8:2, v:v) were added to 4 ml of sample. After vortex mixing (1 min) and centrifugation (3400 rpm, 20 min) the upper organic layer was transferred to a clean test-tube and evaporated to dryness (N_2, 35 °C). Extraction efficiency for diazepam and nitrazepam was greater than 97 %. The residue was reconstituted in 75 µl methanol and 20 µl was injected into the HPLC system. Calibration curves were constructed over the range of 5 to 150 ng ml^{-1} and the intra-day coefficient of variation (n = 5) ranged between 8.23 % (5 ng ml^{-1}) and 5.22 % (150 ng ml^{-1}).

The HPLC system consisted of a Kontron analytic LC 410 Pump (Zurich, Switzerland) which delivered mobile phase (acetonitrile: water with 1 % triethylamine adjusted to pH 3 with 85 % orthophosphoric acid; 50:50, v:v) at a flow rate of 1.5 ml min^{-1}, to a C_{18} chromatographic column (Spherisorb, S10, ODS1). Samples were injected using a 7010 Rheodyne manual injector (Cotati, CA, USA) and column effluent was monitored using a Waters Lambda-max model 481 absorbance detector (Milford, USA) set to 254 nm. The retention time of diazepam and nitrazepam was 8.5 and 4.5 min, respectively. Known metabolites of diazepam (oxazepam, temazepam and desmethyldiazepam; Sigma) eluted at 5.2, 5.7 and 6.5 min, respectively.

Hepatic perfusion. — The single-pass isolated perfused *in situ* rat liver preparation using male Sprague-Dawley rats (200-400 g), was essentially that described previously (6, 10). The perfusate was freshly prepared and filtered (0.2 µm) Krebs-Bicarbonate buffer containing 3 g l^{-1} of D(+)-glucose and 10 mg l^{-1} sodium taurocholate (Sigma), equilibrated to pH 7.4 with humidified O_2/CO_2 (95/5) and maintained at 37 °C. Perfusate was delivered to the liver via a cannula inserted into the hepatic portal vein and liver outflow was collected via a cannula inserted into the vena cava through the right atrium. In each case, the liver was stabilized for 15 to 20 min using drug and protein-free perfusate. At the end of the experiment, the liver was removed and weighed. Diazepam did not bind to the perfusion apparatus.

Viability of the liver preparation and linearity of diazepam elimination. — Preliminary experiments (n = 4) were conducted to assess the viability of the perfused liver preparation. In each preparation, the perfusate was alternated between 0 and 1 % (g/100 ml) HSA for 120 min after the stabilization period, while the total concentration of diazepam was maintained at 1 mg l^{-1}. Hepatic outflow samples were obtained every 5 min and analysed for diazepam. For these and subsequent experiments, the condition of the liver during perfusion was also assessed by monitoring the bile output and the recovery of the inflowing perfusate, and by visual examination of the liver.

Linearity with respect to diazepam elimination was tested with HSA-free perfusate containing 3 different diazepam concentrations (0.5, 1 and 5 mg l^{-1}) perfused in random order for 20 min each.

Experimental design. — In these experiments (n = 4), 50 µl of a solution containing 0.125 µCi of ^{14}C-diazepam in 1 % HSA was rapidly injected into the hepatic portal vein during constant perfusion with unlabelled diazepam (1 mg l^{-1}) in 1 %

HSA. The total effluent from the liver was automatically collected at 2 intervals using a motor driven carousel with 57 sampling holes, and thereafter (into silanized test tubes) at increasing intervals of time for up to 4 min. Perfusate samples were also collected before and after the bolus injection in order to determine the availability at steady state of unlabelled diazepam. The concentration of ^{14}C-diazepam in effluent samples was determined by radiochemical analysis and that of unlabelled diazepam by HPLC.

Data analysis. — Under linear conditions, the outflow profile of a substance injected into the hepatic portal vein is given by:

$$C(t) = \frac{D}{Q}\, x(t) * w(t)_{NH} * w(t)_{H} \qquad \text{[Eq. 1]}$$

where C(t) is the concentration of material in the hepatic outflow at time t, D is the amount of drug administered, Q is the hepatic blood (perfusate) flow rate, x(t) is the function that describes the form of drug input (bolus, constant infusion, etc.), $w(t)_{NH}$ and $w(t)_{H}$ are the unit impulse-responses for the non-hepatic and hepatic regions of the experimental system, respectively, and * denotes the convolution integral.

By expressing output as a fraction of the dose appearing per unit time (frequency output), y(t), and on taking Laplace transforms, Eq. 1 becomes (when the substance is injected as a bolus, x(s) is equal to unity):

$$y(s) = w(s)_{NH} \cdot w(s)_{H} \qquad \text{[Eq. 2]}$$

where $w(s)_{NH}$ and $w(s)_{H}$ are the transfer functions for non-hepatic and hepatic regions of the experimental system, respectively.

According to the theory of the axial dispersion model, when a substance is injected into the liver and distributed at a finite rate between the vascular and cellular spaces (two-compartment dispersion model), the concentration of the substance in blood (perfusate), C_B, as a function of time (t) and distance along the liver length (z), assuming elimination from the cellular space, is given by (15, 21):

$$\frac{\delta C_B}{\delta t} = D\,\frac{\delta^2 C_B}{\delta z^2} - v\,\frac{\delta C_B}{\delta z} - k_{12}\, C_B + K_{21}\, C_c \qquad \text{[Eq. 3]}$$

The corresponding mass balance equation for the cellular compartment is

$$\frac{\delta C_c}{\delta t} = \frac{V_B}{V_C}\, K_{12}\, C_B - k_{21}\, C_c - k_{23}\, C_c \qquad \text{[Eq. 4]}$$

Where D is the axial dispersion coefficient, which characterises the degree of axial dispersion of the injected substance in blood, v is the linear velocity of blood, k_{12} and k_{21} are the first-order rate constants for the transfer of solute into and out of the cellular space, respectively, k_{23} is the first-order rate constant for the irreversible removal of solute from the cellular compartment, V_B and V_C are the volumes of the vascular and cellular spaces, respectively, and C_c is the concentration of drug in the cell.

Equations 3 and 4 comprise a set of second order partial differential equations. Assuming mixed boundary conditions (see discussion), the Laplace transforms equations defining the one and two compartment forms of the dispersion model, respectively, are (15, 21):

$$y(s) = w(s)_{NH} \cdot \exp\left[\frac{1 - [1 + 4\, D_n\, (R_N + \frac{V_H}{Q}\, s)]^{1/2}}{2\, D_N}\right] \qquad \text{[Eq. 5]}$$

$$y(s) = w(s)_{NH} \cdot \exp\left[\frac{1 - [1 + \frac{4 V_B D_N}{Q}(s + k_{12} - \frac{k_{12} k_{21}}{s + k_{21} + k_{23}})]^{1/2}}{2 D_N}\right] \quad \text{[Eq. 6]}$$

where V_H is the volume of distribution of the drug in the liver and D_N, dispersion number, represents the degree of axial dispersion of the injected substance in blood. The term R_N (the efficiency number) is given by:

$$R_N = \frac{CL_{int} \cdot fu \cdot \rho}{Q} \quad \text{[Eq. 7]}$$

where intrinsic clearance, CL_{int}, is defined as the proportionality constant between the rate of elimination and the unbound substrate concentration within the cell, and ρ is given by:

$$\rho = \frac{P}{P + CL_{int}} \quad \text{[Eq. 8]}$$

where P is the permeability of the hepatocyte membrane to the drug.
CL_{int} may also be estimated using the following equation (14):

$$CL_{int} = \frac{k_{12} \cdot k_{23} V_B}{K_{21} fu} \quad \text{[Eq. 9]}$$

After bolus input into the hepatic portal vein, the concentration of ^{14}C-diazepam in the hepatic outflow at time t, C(t), was expressed as a fraction of the dose appearing per second, y(t), using the following expression:

$$y(t) = \frac{C(t)\, Q}{D} \quad \text{[Eq. 10]}$$

where Q is the perfusate flow rate and D is the injected dose. Availability was taken to be the area under the frequency outflow versus mid-point time profile, calculated using the trapezoidal rule with extrapolation to infinity. In all cases, the extrapolated area accounted for less than 2 % of the total area.

Equations 5 and 6 were fitted to the experimental frequency output versus time profiles using a numerical inversion programme (MULTI-FILT, version 2.0), (21, 22), which couples the FILT algorithm to a multiple non-linear regression package, with a weighing scheme of $1/y(t)_{observed}$. However, it was first necessary to determine the transfer function for the non-hepatic regions of the experimental apparatus, $w(s)_{NH}$, by applying the following equation

$$w(s)_{NH} = \exp\left[\frac{1 - (1 + 4 D_{N,NH} \cdot MRT_{NH} \cdot s)^{1/2}}{2 D_{N,NH}}\right] \quad \text{[Eq. 11]}$$

to the frequency output profile of ^{14}C-diazepam in the absence of a liver. This analysis provided reproducible estimates of MRT_{NH} (5.3 s) and $D_{N,NH}$ (0.041). These values were substituted into equation 10 which was then used to define $w(s)_{NH}$ in Eqs. 5 and 6. V_B was assumed to represent the hepatic distribution volume for albumin, which is confined to the blood space and the space of Disse. This volume has been found to represent about 15 % of wet liver weight (8, 16, 19).

Results

Viability of the liver preparation and linearity. — Fig. 1 shows a representative plot of availability versus time when diazepam was perfused at a constant input rate (1 mg l^{-1}; 15 ml min^{-1}) while alternating between protein-free perfusate and perfusate containing 1 % HSA. In all cases (n = 4), the availability of diazepam did not change by more than 10 % throughout the experiment for each condition.

Experiments conducted in two perfused

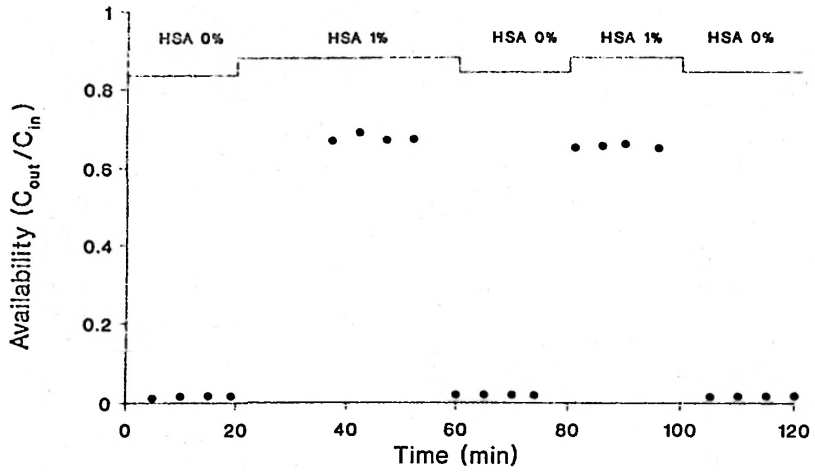

Fig. 1. *Availability of diazepam versus time in a representative liver while alternating between protein-free perfusate and perfusate containing 1 % HSA with constant rate drug infusion (1 mg l^{-1}; 15 ml min^{-1}).* The design of the experiment is also shown.

livers indicated that the availability of diazepam did not change with drug concentration up to 5 mg l^{-1}. In one experiment, F was 0.0089 at 0.5 mg l^{-1}, 0.0085 at 1 mg l^{-1} and 0.0090 at 5 mg l^{-1}, and in the other experiment F was 0.0162, 0.0141 and 0.0160 at 0.5, 1 and 5 mg l^{-1}, respectively.

Protein binding determination. — The fraction of diazepam unbound in fresh perfusate at different HSA concentrations is shown in figure 2. At each HSA concentration, the binding of diazepam in hepatic outflow samples was almost identical to that determined in fresh perfusate suggesting that binding was not influenced by passage through the liver. The unbound fraction of diazepam in protein-free perfusate collected from the effluent of different liver preparations (n = 5) was 1.000 ± 0.031, indicating that the drug did not bind to material escaping from the liver into the perfusate during the experiment.

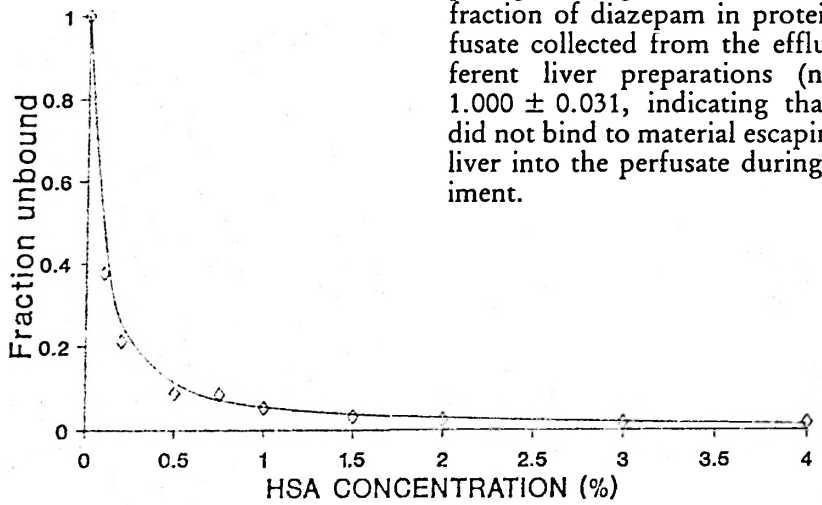

Fig. 2. *Effect of albumin concentration in the perfusate on the fraction unbound of diazepam.*

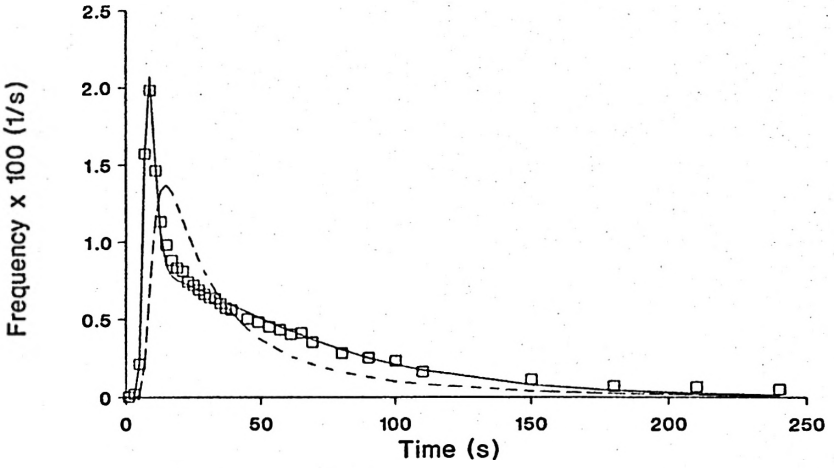

Fig. 3. *Typical frequency outflow versus time profile for diazepam and the relationship predicted from the application of the one-compartment (---) and two-compartment (—)* dispersion models.

Impulse-response experiments. — Fig. 3 shows a representative frequency outflow versus time profile for diazepam together with the profiles predicted from the one- and two-compartment dispersion models (Eqs. 5 and 6, respectively). In all cases, the outflow profile for diazepam appeared as a sharp peak which eluted over the first 25 second interval, followed by a slowly eluting tail. The availability of ^{14}C-diazepam, estimated from the area under the frequency outflow versus mid-point time profile, was 0.67 ± 0.04. The availability of diazepam, estimated as the ratio of C_{out}/C_{in} for unlabelled material using samples collected immediately before and after the bolus injection, was 0.68 ± 0.03. The identical availability estimates for diazepam determined using non-specific (radiochemical) and specific (HPLC) methods suggests that the metabolites of ^{14}C-diazepam did not contribute to the total radioactivity in hepatic outflow.

The one-compartment dispersion model could not adequately describe the outflow profile of ^{14}C-diazepam (see, for example, fig. 3). In contrast, the outflow profile was well described by the two-compartment dispersion model, providing estimates for D_N, k_{12}, k_{21} and k_{23} (table I) with a high degree of precision (coefficient of variation for the parameter estimate <

Table I. *Parameters obtained by applying the two-compartment dispersion model to the outflow profile for diazepam after bolus injection into the hepatic portal vein at 15 ml min^{-1}.*

Experiment	D_N	CL_{int} (ml min^{-1})	k_{12} (s^{-1})	k_{21} (s^{-1})	k_{23} (s^{-1})
1	0.301	194	0.6572	0.0513	0.0062
2	0.350	178	0.7213	0.0739	0.0076
3	0.212	84	0.8509	0.0668	0.0048
4	0.142	83	0.9441	0.0620	0.0026
mean ± S.D.	0.251 ± 0.093	135 ± 59	0.7934 ± 0.1288	0.0635 ± 0.0095	0.0053 ± 0.0021

10 %). The intrinsic clearance of diazepam (table I) was estimated in each case using these first-order rate constants, the fu of diazepam at a HSA concentration of 1 % (0.052), and V_B (15 % of liver weight) (Eq. 9).

Discussion

In these experiments, a radiolabelled tracer dose of diazepam was injected as a bolus into the hepatic portal vein during a constant infusion of unlabelled diazepam (1 mg l^{-1}, 15 ml min^{-1}). The fact that the availabilities determined from radiochemical analysis of ^{14}C-related material and HPLC analysis of unlabelled diazepam were almost identical suggests that metabolites or metabolic byproducts of ^{14}C-diazepam did not contribute to the total radioactivity in the hepatic outflow. The outflow profile for ^{14}C-diazepam was well described by the two-compartment form of the axial dispersion model, in which radial distribution is assumed to proceed at a finite rate. Similar findings have been reported for diclofenac, oxacillin, and ampicillin (6, 23). Diazepam, in the presence of 1 % HSA, is highly bound ($\approx$ 95 %) within the perfusate. This could be the reason why there is a sharp peak in the outflow profile followed by a slowly eluting tail. Such profile is in contrast to that predicted by the one-compartment dispersion model (fig. 3), which assumes instantaneous radial transfer of a substance between the vascular and cellular spaces. The peak may, therefore, represent injected material which did not enter the cellular space during passage through the liver (throughput component) because of extensive binding to HSA, which is confined to the extracellular space. The tail may be produced by the material returning from the cellular space having escaped elimination (returning component). Although, it could be argued that the initial peak might also be due to a hepatocyte membrane permeability limitation, a permeability problem *per se* seems extremely unlikely because P (estimated as $k_{12} \cdot V_B/fu$) had a value of 1626 $\pm$ 343.8 ml min^{-1}, which is more than 100 times that of Q. The estimated value for P is also more than 10 times that of CL_{int}. Hence, diazepam hepatic elimination is perfusion limited rather than capacity limited. The value estimated for D_N is of the same order as that estimated for diclofenat (6) and other compounds (15, 16), using impulse-response techniques in the isolated perfused rat liver.

It should be noted that, in this study, the solution of the dispersion model obtained by assuming mixed boundary conditions (Eqs. 5 and 6) has been used. Although the closed boundary conditions may be more appropriate mathematically, the choice of boundary conditions is relevant only at high dispersion numbers (15) and the use of the mixed boundary conditions was adopted because of its relative mathematical simplicity, particularly when applied to transient data.

The magnitude of D_N (overall range of 0.2-0.5) is similar to that reported for substances which are restricted to the vascular and extracellular spaces (15, 16) such as erythrocytes and albumin, providing support to the idea that the dispersion number is a characteristic of the liver and relatively independent of the substance, and suggesting that the axial spreading of diazepam in the perfused rat liver is determined primarily by the heterogeneity of the flow in the liver. However, it should be noted that a much higher D_N may be needed to account for data on the hepatic elimination of taurocholate (5, 18), lignocaine and pethidine (1) under steady-state conditions. Data for these substrates appear to conform closely to the predictions of the venous equilibrium model, possibly as a consequence of processes such as axial diffusion within hepatic tissue (12), protein facilitated transfer of substrate across an unstirred fluid

layer adjacent to the hepatocyte surface or transverse enzyme heterogeneity (15).

Resumen

Se evalúa la consistencia del modelo de dispersión axial de eliminación hepática utilizando experimentos de impulso-respuesta con diacepam en hígado de rata aislado y perfundido. El modelo de dispersión de dos compartimentos se ajusta a los datos de concentración de diacepam en el eluyente hepático tras la administración de diacepam marcado en la vena porta (n = 4). Los valores estimados de D_N y CL_{int} son 0,251 ± 0,093 y 135 ± 59 ml min^{-1}, respectivamente. El modelo de dispersión de un compartimento, que presupone que la distribución radial de una sustancia a los espacios accesibles en el hígado es instantánea, no es adecuado para describir la distribución de los tiempos de permanencia del diacepam en el hígado. El valor de D_N, un parámetro estadístico que caracteriza la dispersión axial de las sustancias durante su paso a través del hígado, es similar al determinado para otras sustancias como eritrocitos, albúmina, sucrosa y agua. Estos resultados sugieren que la dispersión del diacepam en el hígado aislado y perfundido de rata viene determinada principalmente por la estructura de la microvasculatura hepática.

Palabras clave: Diacepam, Eliminación hepática, Modelos fisiológicos, Modelo de dispersión, Hígado de rata perfundido y aislado.

References

1. Ahmad, A. B., Bennett, P. N. and Rowland, M.: *J. Pharmac. Pharmacol.*, 35, 219-224, 1983.
2. Bass, L., Robinson, P. and Bracken, A. J.: *J. Theor. Biol.*, 72, 161-184, 1978.
3. Bass, L., Roberts, M. S. and Robinson, P. J.: *J. Theor. Biol.*, 126, 457-482, 1987.
4. Bass, L. and Pond, S. M.: In «Pharmacokinetics: Mathematical and statistical approaches to metabolism and distribution of chemicals and drugs». (Pecile, A. and Rescigno, A., eds.). Plenum Publishing Corp., London, 1988, pp. 245-269.
5. Ching, M. S., Morgan, D. J. and Smallwood, R. A.: *J. Pharm. Exp. Ther.*, 250, 1048-1054, 1989.
6. Evans, A. M., Hussein, Z. and Rowland, M.: *J. Pharm. Pharmacol.*, 43, 709-714, 1991.
7. Forker, E. L. and Luxon, B.: *Am. J. Physiol.*, 235, E648-E660, 1978.
8. Goresky, C. A.: *Am. J. Physiol.*, 204, 626-640, 1963.
9. Pang, K. S. and Rowland, M.: *Pharmacokin. Biopharm.*, 5, 625-653, 1977.
10. Pang, K. S. and Rowland M.: *J. Pharmacokin. Biopharm.*, 5, 655-680, 1977.
11. Raisys, V. A., Friel, P. N., Graaff, P. R., Opheim, K. E. and Wilensky, A. J.: *J. Chromatogr.*, 183, 441-448, 1980.
12. Rivory, L. P., Roberts, M. S., Pond, S. M.: *J. Pharmacokin. Biopharm.* 20: 19-61, 1992.
13. Roberts, M. S. and Rowland, M.: *J. Pharm. Sci.*, 74, 585-587, 1985.
14. Roberts, M. S. and Rowland, M.: *J. Pharmacokin. Biopharm.*, 14, 227-260, 1986.
15. Roberts, M. S., Donaldson, J. D. and Rowland, M.: *J. Pharmacokin. Biopharm.*, 16, 41-83, 1988.
16. Roberts, M. S., Fraser, S., Wagner, A. and McLeod, L.: *J. Pharmacokin. Biopharm.*, 18, 209-234, 1990.
17. Sawada, Y., Sugiyama, Y., Miyamoto, Y., Iga, T. and Hanano, M.: *Chem. Pharm. Bull.*, 33, 319-326, 1985.
18. Smallwood, R. H., Morgan, D. J., Mihaly, G. W., Jones, D. B. and Smallwood, R. A.: *J. Pharmacokin. Biopharm.*, 16, 377-396, 1988.
19. Tsao, S. C., Sugiyama, Y. Sawada, Y., Nagase, S., Iga, T. and Hanano, M.: *J. Pharmacokin. Biopharm.*, 14, 51-64, 1986.
20. Wilkinson, G. R.: *Pharm. Rev.*, 39, 1-47, 1987.
21. Yano Y., Yamaoka, K., Aoyama, Y. and Tanaka, H.: *J. Pharmacokin. Biopharm.*, 17, 179-202, 1989.
22. Yano, Y., Yamaoka, K. and Tanaka, H.: *Chem. Pharm. Bull.*, 37, 1535-1538, 1989.
23. Yano, Y., Yamaoka, K., Minamide, T., Nakagawa, T. and Tanaka, H.: *J. Pharm. Pharmacol.*, 42, 632-636, 1990.