J. Pharm. Pharmacol. 1998, 50: 865–870
Received January 7, 1998
Accepted April 3, 1998

Relative Dispersions of Intra-albumin Transit Times across Rat and Elasmobranch Perfused Livers, and Implications for Intra- and Inter-species Scaling of Hepatic Clearance using Microsomal Data

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Abstract

It is recognized that vascular dispersion in the liver is a determinant of high first-pass extraction of solutes by that organ. Such dispersion is also required for translation of in-vitro microsomal activity into in-vivo predictions of hepatic extraction for any solute. We therefore investigated the relative dispersion of albumin transit times (CV^2) in the livers of adult and weanling rats and in elasmobranch livers.

The mean and normalized variance of the hepatic transit time distribution of albumin was estimated using parametric non-linear regression (with a correction for catheter influence) after an impulse (bolus) input of labelled albumin into a single-pass liver perfusion. The mean ± s.e. of CV^2 for albumin determined in each of the liver groups were $0{\cdot}85 \pm 0{\cdot}20$ ($n = 12$), $1{\cdot}48 \pm 0{\cdot}33$ ($n = 7$) and $0{\cdot}90 \pm 0{\cdot}18$ ($n = 4$) for the livers of adult and weanling rats and elasmobranch livers, respectively.

These CV^2 are comparable with that reported previously for the dog and suggest that the CV^2 of the liver is of a similar order of magnitude irrespective of the age and morphological development of the species. It might, therefore, be justified, in the absence of other information, to predict the hepatic clearances and availabilities of highly extracted solutes by scaling within and between species livers using hepatic elimination models such as the dispersion model with a CV^2 of approximately unity.

The availability of solutes is dependent on first-pass extraction (E) of solutes in the liver. Roberts & Rowland (1985, 1986) and Roberts et al (1988) have shown that high extraction is inappropriately described by either of the classical well-stirred or tube hepatic elimination models and suggest that the blood-flow-, protein-binding- and enzyme-activity-dependence of highly extracted drugs must account for intrinsic clearance by the liver and the heterogeneity of blood-flow distribution in the liver. Two parameters, the efficiency number (determined by the intrinsic metabolic or biliary clearance of the liver, fraction of solute unbound in the blood and blood flow) and dispersion number D_N (determined by the vascular dispersion of a tracer, CV^2, and defined by the out-flow concentration–time profile after bolus administration to the liver), were used to predict hepatic extraction for solutes under a range of conditions. In general, Roberts and Rowland expressed their results in terms of hepatic availability, F, the fraction of solute escaping extraction during the first pass through the liver.

The dispersion model has now been developed in several respects (Rowland & Evans 1991), including studies on the dynamics of distribution with a resultant two-compartment dispersion model (Roberts et al 1988; Yano et al 1989), non-linear pharmacokinetics (Roberts et al 1989), the effect of flow and perfusate content on the dispersion number (Roberts et al 1990a), retrograde perfusions (Roberts et al 1990b), tissue diffusion (Rivory et al 1992) and the effect of lipophilicity on hepatic dispersion and distribution (Chou et al 1993). Of particular relevance to this study is the use of a dispersion model to interrelate in-vitro microsomal

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enzyme activity and whole-organ hepatic elimination kinetics (Roberts & Rowland 1986). The CV^2 defined by rat liver dispersion-model analysis has been used in intra- and inter-species scaling of in-vitro hepatic metabolism into in-vivo hepatic first-pass extraction (Fuse et al 1995; Suzuki et al 1995). A key question in applying the dispersion model to such analyses is—what is the magnitude of the dispersion number D_N (or the vascular dispersion CV^2)? This parameter is usually unknown in the translation of microsomal enzyme activity or hepatocyte activity for the liver of a given species at a stage of development in the prediction of the equivalent hepatic availability.

In this study, we determined the albumin dispersion of the liver of the adult rat, the weanling rat and the sting-ray (an elasmobranch) using the impulse-response technique with albumin as the marker. The weanling rat was chosen to enable examination of the effect of intra-species development by use of the smallest liver we were able to perfuse reproducibly. The sting-ray liver was chosen as an example of a liver from a species less developed morphologically. The sting-ray's lack of serum albumin has previously been used to enable understanding of the role of proteins in the transport of solutes in the mammalian liver (Weisiger et al 1984). The CV^2 found for the three preparations was of a similar order of magnitude, providing some support for the use of a constant CV^2 in the translation of microsomal availability into in-vivo availability (Fuse et al 1995; Suzuki et al 1995; Iwatsubo et al 1997).

Materials and Methods

Liver perfusion

The cannulation, perfusion and conduct of impulse-response studies in fed adult female Sprague-Dawley rats, approximately 190–210 g, (liver weight 6·1–8·5 g) was similar to that described in earlier studies (Roberts et al 1990a, b). Flow rates of 15 and 30 mL min^{-1} were used for Krebs-Henseleit buffer containing 0·25% taurocholic acid and 1·0% bovine serum albumin. The injectate (50 μL) in perfusate contained [^{125}I]bovine serum albumin or Evans Blue, with out-flow fractions being collected for 1 or 2 s over 2 min after bolus (impulse) input of injectate. Samples of [^{125}I]albumin were analysed by gamma counting and Evans Blue by spectrophotometry (Roberts et al 1990a, b). The weanling rats were 3-week fed female Sprague-Dawley rats, 55–70 g (liver weight 1·9–2·6 g); the flow rate was 7·5 or 15 mL min^{-1} using the procedure described for adult rats but with collection intervals of 2·5 or 4 s up to 3 min.

The elasmobranch (sting-ray studies) were conducted on the species *Dasyatis kuhlii* (blue spot sting-ray), usually within 24 h of capture from the waters in and around Moreton Bay, Queensland, Australia. The barbs were removed, the fish anaesthetized by intraperitoneal administration of pentobarbitone, a midline laparomoty undertaken, pancreas clamped off and the pericardium exposed. The portal vein was cannulated and slow perfusion with an elasmobranch Ringer solution (Reed et al 1982) commenced. The conus arteriosus (fourth chamber of the heart) was then cannulated. Flow rates of 30 or 60 mL min^{-1} were used in all liver and catheter studies. The liver weights ranged from 9·9–23·5 g. The injectate in the perfusate (50 μL) contained Evans Blue; out-flow fractions were collected at 1-s intervals.

Catheter perfusion

To separate the effects of the catheters on the impulse-response profiles, impulse-response studies were also undertaken exactly as described for each of the studies above but using the catheters only, i.e. with the liver absent. The resulting profiles are referred to as catheter-density functions.

Data analysis

The concentration of albumin C(t) in a given out-flow sample after bolus administration of dose D into the isolated perfused liver plus catheters with flow Q was expressed as out-flow fraction mL^{-1} (concentration in sample/dose of indicator). The out-flow fraction mL^{-1} for the catheter was deduced in a similar manner after bolus administration of labelled albumin into catheters alone. The catheter function was then fitted by either a single inverse Gaussian distribution with a lag-time or, for the elasmobranch livers, a single exponential function with a lag-time using the non-linear regression program and Scientist (MicroMath Scientific Software, Salt Lake City, UT) and a weighting of $1/y_{obs}$. C(t) can be described as a convolution (denoted by the symbol *) of the unit impulse response curves of the catheter system $f_{Cath}(t)$ and the liver $f_{Liv}(t)$:

$$C(t) = (D/Q)f_{Cath}(t)*f_{Liv}(t) \qquad (1)$$

because these are subsystems of the perfusion arranged in series. Estimation of $f_{Liv}(t)$ can be made by deconvoluting C(t) using the assumed function for $f_{Cath}(t)$ and the parameter values derived from the catheter profiles as described earlier as fixed values. Of particular interest in this analysis are the first and second moments of $f_{Liv}(t)$ obtained when albumin is injected as a vascular marker. The mean

transit time MTT, the first moment of $f_{Liv}(t)$, is defined by:

$$MTT = V/Q = \int_0^\infty tf_{Liv}(t)dt \quad (2)$$

where V is the volume of albumin space in the liver. The second moment of $f_{Liv}(t)$ is the variance of transit-time distribution (VTT):

$$VTT = \int_0^\infty t^2 f(t)dt - MTT^2 \quad (3)$$

VTT is frequently normalized with the MTT to yield the normalized variance or vascular dispersion CV^2:

$$CV^2 = VTT/MTT^2 \quad (4)$$

An underlying relationship $CV^2 = 2D_N$, where D_N is the dispersion number defined by Roberts & Rowland (1985), holds for unit-impulse-response curves of intravascular indicators which can be described by an inverse Gaussian density function or dispersion model with mixed boundary conditions (Roberts et al 1988). The dispersion number for the adult rat liver has previously been estimated from the normalized variance using numerical integration by recognizing the additivity of the mean transit times and variances of transit times for the catheters and liver (Roberts et al 1990a).

As CV^2 is highly dependent on the extrapolation of the VRT to time infinity, CV^2 was estimated from parameters derived from equation 1 as described by Weiss & Roberts (1996) using the normalized out-flow concentration-time profile, C(t), of the albumin reference in the liver plus catheters after bolus administration and non-linear regression in the Laplace domain (Weiss & Roberts 1996). The function for the liver $f_{Liv}(t)$ was assumed to be an empirical parametric model described by a sum of two inverse Gaussian density functions. The parameters for $f_{Liv}(t)$ were then deduced from C(t) and the assumed catheter function $f_{Cath}(t)$ with fixed parameters (estimated in separate non-linear regression of the catheter profiles alone) by deconvolution in the Laplace domain using the non-linear regression program packages Minim (Purves 1995) and Scientist (MicroMath Scientific Software, Salt Lake City, UT). The data were weighted according to $1/y_{obs}$ except when poor fits were obtained for the tail of the curves, when a weighting scheme $1/y_{obs}^2$ was used.

Results and Discussion

Representative concentration–time profiles for albumin for the adult and weanling rat and sting-ray livers are shown in Figure 1. The data are better fitted with an empirical function (a mixture of two

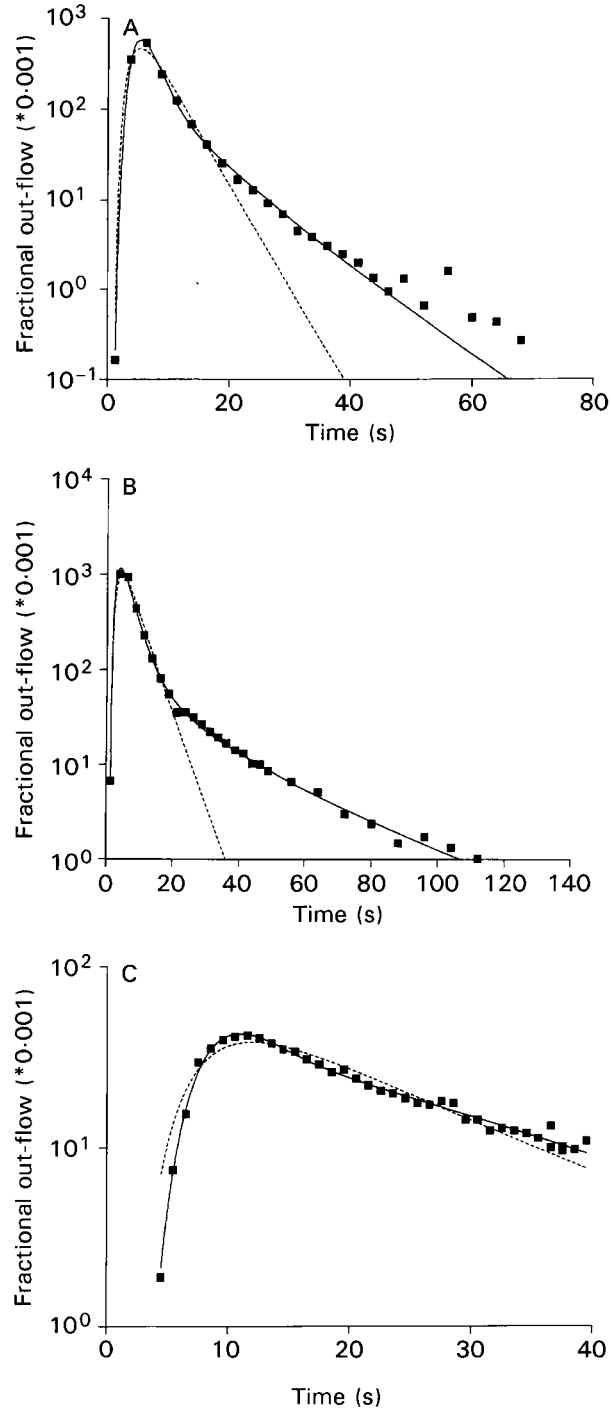

Figure 1. Representative fits of a mixture of two inverse Gaussian density functions (solid line) and a single inverse Gaussian (dashed line) to out-flow concentration–time profiles for albumin: (A) adult rat liver 9406b; (B) weanling rat liver 9401d; (C) elasmobranch (sting-ray) liver 95s4.

inverse Gaussians, solid line) convolved with the catheter function, than the usual dispersion model (or single inverse Gaussian distribution, dotted line) convolved with the catheter function. The dispersion model seems to describe poorly the tail components of the out-flow concentration-time profiles. Also apparent in Figure 1 is random scatter of out-flow fractions at the later times. It was this scatter and the resulting uncertainty in extrapolating out-flow concentrations to time infinity which led to use of a parametric approach for estimation of moments. The regression fit provided by the double-inverse Gaussian was satisfactory for all groups. We had previously found that this function was the most suitable for the description of out-flow concentration-time profiles in the perfused hind limb (Weiss & Roberts 1996).

Table 1 summarizes the values of CV^2 and V obtained for each perfusion. The CV^2 obtained for each group of livers were (mean ± s.e.): adult rat 0·85 ± 0·20 (n = 12), weanling rat 1·48 ± 0·33 (n = 7) and sting-ray 0·90 ± 0·18 (n = 4). It is also apparent that the sting-ray liver is substantially larger than that of the adult rat, the albumin volumes being 49·36 ± 37·21 mL and 2·90 ± 0·28 mL, respectively. The weanling rat liver is substantially smaller than that of the adult rat, as indicated by an albumin volume of 1·37 ± 0·21 mL, compared with the adult rat liver albumin volume of 2·90 ± 0·28 mL. No significant differences in the median values could be detected among the groups ($P = 0·145$, Kruskal-Wallis analysis of variance of ranks). Pang et al (1988) and Cheung et al (1996) have shown that liver vascular volume and water volume are flow-related, higher flows leading to an increase in capillary pressure and albumin volume.

The fixed-catheter parameters used in the convolutions were dependent both on flow-rate and on type of liver. For instance, the catheter associated with the rat liver yielded parameter values of MTT 0·39 s, CV^2 1·14 and lag-time 0·49 s at 10 mL min^{-1} and MTT 0·21 s, CV^2 0·89 and lag-time 0·20 s at 20 mL min^{-1}. The MTT for the elasmobranch catheters was 1·51 s. It is to be noted that the MTT for the catheters is, on average, an order of magnitude less than that for the livers and only exerts a limited influence on the observed profiles for C(t). However, in this analysis, the potential effects of the catheters have been accounted for by including their contribution in the fitting procedure used to determine $f_{Liv}(t)$.

Table 1. Derived albumin dispersion CV^2 and liver albumin volume V for isolated perfused livers from adult rats (flow rate 18–32 mL min^{-1}), weanling rats (flow rate 7·5–15 mL min^{-1}) and sting-ray livers (flow rate 30–40 mL min^{-1}) on the basis of the out-flow concentration–time profiles after bolus injections of labelled albumin.

Liver	CV^2	V (mL)
Adult rat	0·85 ± 0·20	2·90 ± 0·28
Weanling rat	1·48 ± 0·33	1·37 ± 0·21
Sting-ray	0·90 ± 0·18	49·36 ± 37·21

Values are means ± s.d.

Figure 2 shows photographs of histological sections of livers from each group studied. Each liver

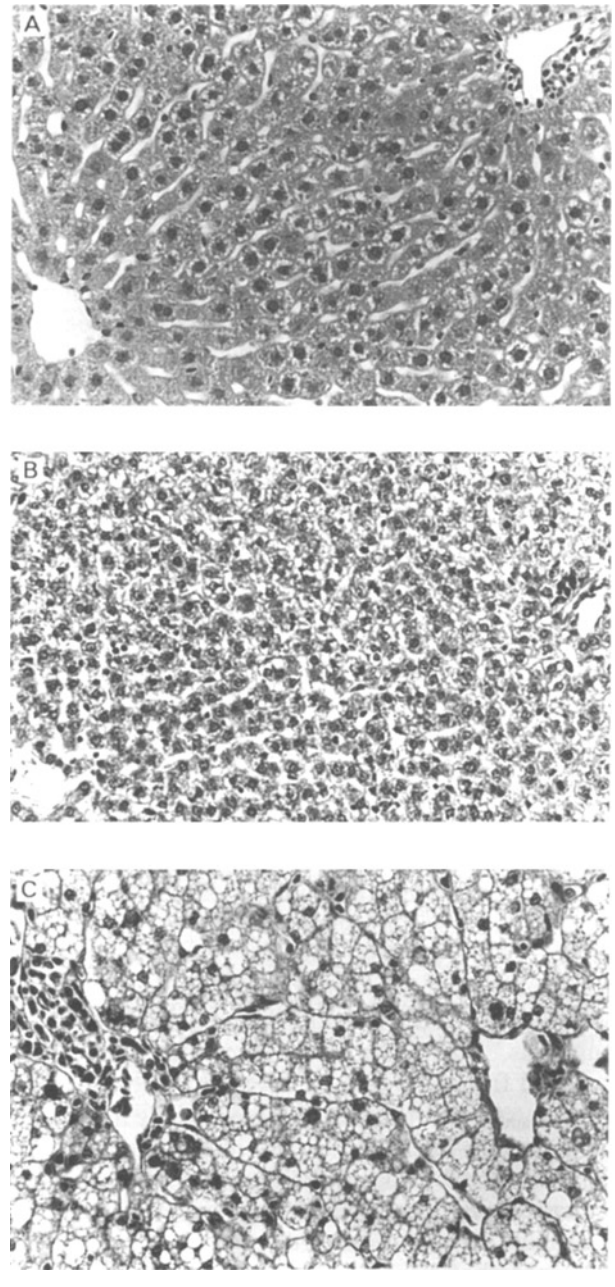

Figure 2. Representative micrographs of histological sections taken from (A) adult rat liver; (B) weanling rat liver; (C) elasmobranch (sting-ray) liver. The figures are orientated with portal venule at the top and hepatic venule at the bottom (Magnification 295×).

section micrograph is orientated with portal venule at the top and hepatic venule at the bottom. The sinusoidal (microvasculature) arrangement in each liver appears similar, with the most notable differences being the smaller cells and higher mitotic activity in the weanling rats and the somewhat different architecture of hepatocytes from the elasmobranch liver, as evidenced by their columnar and fatty appearance. It is evident that the vascular anatomy of the livers is sufficiently similar to yield CV^2 of similar order for the different livers (Table 1). Roberts & Rowland (1985) had previously suggested that the basic dispersion process in the liver is defined by "the branching of the sinusoids and interconnections, together with variations in the velocity and path lengths travelled by elements of blood". Most striking appears the result that the median CV^2 of 0·90 measured for the sting-ray liver is not significantly different from the values 0·85 and 1·48 measured for adult and weanling rats, respectively. Hepatic function in a marine elasmobranch fish such as the sting-ray differs from that in the rat in terms of hepatic vein sphincters (Johansen & Hanson 1976) and the absence of serum albumin in the elasmobranch (Weisiger et al 1984).

Because a CV^2 near unity reflects an efficient mixing process (Weiss 1995), the current work suggests that systems for optimum intrahepatic solute mixing have been developed in the cartiliginous verterbrates. Also within one species the relative dispersion of CV^2 remains more or less unaffected by anatomical changes because of liver development (weanling compared with adult). In quantitative terms, the CV^2 value for the adult rat (median 0·55) determined by fitting a mixture of inverse Gaussians (Weiss & Roberts, 1996) to the [^{125}I]BSA data is in good agreement with the value of 0·60 estimated by numerical integration from Evans Blue data (Roberts et al 1990a) and the value of 0·66 obtained from a homologous series of barbiturates (Chou et al 1993).

As recently pointed out by Suzuki et al (1995), liver models such as the dispersion model might under some conditions represent a useful approach for intra- and inter-species scaling of hepatic metabolism. Thereby, the amount of intrasinusoidal mixing, as reflected by the relative dispersion of transit times, CV^2, of an intravascular reference (or the dispersion number D_N) is, for highly-extracted drugs, one of the basic factors underlying the prediction of hepatic extraction (or clearance) from the intrinsic clearance of the drug determined in-vitro, i.e. the translation of hepatocyte or microsomal metabolic rates to extraction in perfused systems (Roberts & Rowland 1986; Fuse et al 1995; Iwatsubo et al 1997). However, the similarity in CV^2 relates only to the vascular dispersion caused by the anatomical arrangement of the hepatic blood vessel and exchanging vessel architecture. In contrast, marked differences exist in the intrinsic clearance of each species and in the metabolic pathways used to detoxify exogenous and endogenous chemicals.

This work, which seems to be one of the first to examine the variation of CV^2 between species, suggests that, to a first approximation, CV^2 is similar for the adult rat and the sting-ray. We have previously reported similar CV^2 for dog livers in-vivo and perfused rat livers (Roberts et al 1988) but had not recognized the importance of the observation in terms of inter-species scaling. The dog liver, rat liver and elamsobranch liver represent a wide spectrum of species-liver differences in terms of both size and morphological development.

The similar values for weanling and adult CV^2 imply that changes in hepatic metabolism with the maturing of a species are likely to be defined by a CV^2 with a similar order of magnitude throughout the life-span of a species, with obvious pharmacokinetic prediction implications for highly extracted solutes.

Acknowledgements

We acknowledge support by a University of Queensland Travel Grant and the Bundesministerium für Forschung und Technologie (M. W.). We also thank the National Health and Medical Research Council of Australia, the Queensland and Northern NSW Lions Kidney and Medical Research Foundation for their support and the University of Queensland's Zoology Department for help in providing the elasmobranch studies. Acknowledgement is also given to Associate Professor N. Walker, Department of Pathology, University of Queensland for his assistance in processing and interpreting the histological sections of the livers studied.

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