Application of the Dispersion Model to Describe Disposition Kinetics of Markers in the Dual Perfused Rat Liver

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ABSTRACT:

The liver receives two blood supplies, portal and hepatic, yet most in situ studies use only portal perfusion. A model based on dispersion principles was developed to provide baseline data of the dual perfused rat liver preparation by characterizing the temporal outflow profiles of noneliminated reference markers (vascular marker, red blood cells; extracellular markers, albumin, sucrose; and intracellular markers, urea, water). The model consists of two components: the common and a specific arterial space operating in parallel. The common space receives all the portal flow and some of the arterial flow; the remaining arterial flow perfuses the specific space. Each space is divided into three subspaces: vascular, interstitial, and intracellular. The extent of axial spreading of solute on passage through the common and specific spaces is characterized by their respective dispersion numbers, \( D_n \). The model was fully characterized by analysis of the outflow data following independent bolus administration into the portal vein and hepatic artery. The model provided a good fit of the data for all reference compounds. The estimate of the fraction of the total space assigned to the specific arterial space varied from 4 to 11%, with a mean value of 9%. The estimated \( D_n \) was always small (<0.25) and tended to be greater for the common space (0.08–0.23) than the specific space (0.05–0.12). However, for each space, there was no significant difference in the \( D_n \) value among all reference markers; this is assumed to arise because all markers are reflecting a common feature, the heterogeneity of the microvasculature.

Despite the liver receiving a dual blood supply, the portal vein (PV) has been the main source of input for studying hepatic disposition using the isolated perfused liver preparation. Nevertheless, this approach is unphysiological in the sense that it excludes the possible contribution from the hepatic artery (HA). Studies concerned with the interaction of these two inputs suggest that both a common and specific space exists: the majority of the sinusoids are the common channels for both streams, whereas a small fraction (approximately 5–10%; Field and Andrews, 1968; Ahmad et al., 1984; Reichen, 1988; Kassissia et al., 1994; Pang et al., 1994; Sahin and Rowland, 1998b) remains specific to the HA. Although existing clearance data suggest that the difference between the PV and HA might be attributable to the presence of the specific arterial space (Ahmad et al., 1984; Pang et al., 1994; Sahin and Rowland, 2000b), they do not provide any information on the disposition characteristics of this space. However, this can be achieved by defining each space in a model.

The axial dispersion model has its origins in chemical engineering (Kreft and Zuber, 1978), where it was developed to describe the mixing of substances on transport through a packed reactor bed. It was introduced into the field of pharmacokinetics by Roberts and Rowland (1986a,b,c) to describe the mixing events in the liver. They proposed that the branch points of the sinusoids in the liver and the interconnections between them enabled convective mixing to occur, and that the variation in path lengths traveled by fractional elements of the solute was a result of this extensive branching. The model is characterized by two main dimensionless parameters: the efficiency number (\( R_n \)) and the axial dispersion number (\( D_n \)). The efficiency number characterizes the overall efficiency of the elimination process. The dispersion number quantifies the degree of axial spreading of a solute on transit through the liver. The axial dispersion model has been successfully applied to the majority of the hepatic outflow data of reference space markers and drug substances from the single PV-perfused liver preparation (Evans et al., 1991, 1993; Diaz-Garcia et al., 1992; Chou et al., 1993; Hussein et al., 1994). However, its application to the outflow data obtained from the dual perfused rat liver preparation is lacking.

We report here the successful application of a dispersion model to the analysis of the hepatic outflow data of reference space markers obtained from the rat liver preparation operating in dual perfusion mode. The proposed model comprises two parallel components (common and specific spaces), each incorporating three reference spaces (vascular, interstitial, and intracellular), with axial dispersion occurring within the vascular space of each component.

Materials and Methods

The Axial Dispersion Model. Axial dispersion, or spreading in general, results from the net effect of several events including variations in the velocity and element path lengths resulting in different resident times for elements traveling along different sinusoids, mixing of blood at the branch points of

ABBREVIATIONS: PV, portal vein; HA, hepatic artery; \( D_n \), dispersion number; MTT, mean transit time; \( V_{tc} \), volume of distribution; RBC, red blood cell.
functions. A weighting function is the (output) response in time to a unit substance, $f(x,t)$, in a region and is an operator used to transform the impulse input function $x(t)$ into the output function $f(x)$. When the substrate is administered as a bolus into the PV, $x(t)_{PV} = 1$.

**Arterial Input.** After arterial administration, the substrate has access to both spaces so that the fractional recovery rate outflow versus time profile (in the Laplace domain) can be written as

\[
f(x)_{HA} = w(s)_{NH} \cdot f(x)_{PV} + w(s)_{sa} + x(s)_{sa} \cdot w(s)_{sa}
\]

where $x(s)_{sa}$ and $w(s)_{sa}$ are the input and transfer functions of the specific arterial space, respectively. When a substrate is administered as a bolus into the HA, $x(t)_{HA} = f_1$ and $x(t)_{sa} = f_2$. It is assumed that the transfer function of the common space $[w(s)_{C}]$ is independent of the route of presentation and is thus defined by the same parameters independent of hepatic input, whereas $w(s)_{sa}$ has its own descriptive parameters.

The transfer functions using the mixed boundary conditions associated with each component of the model are given by the common space:

\[
w(s)_{C} = \exp \left[ \frac{-\sqrt{1 + (4D_{NH}V_{HA} \cdot s)(Q_{PV})}}{2D_{NH}} \right]
\]

and the specific space:

\[
w(s)_{sa} = \exp \left[ \frac{-\sqrt{1 + (4D_{NH}V_{HA} \cdot s)(Q_{PV})}}{2D_{NH}} \right]
\]

where $D_{NH}$ and $V_{HA}$ are the dispersion number and the mean transit time of the substance in the nonhepatic regions, respectively.

**Data Set.** Experimental data obtained from the rat liver preparation operating in dual perfusion mode were used in the present analysis (Sahin and Rowland, 1999, 2000a,b). All experiments were conducted under appropriate Projects and Personal Licenses issued by the UK Home Office. All animals were handled in compliance with Home Office Guidelines. They were kept in a 12-h light/dark cycle temperature-controlled environment and had free access to the drinking water and standard rat diet. Male Sprague-Dawley rats were used as the liver donors. Anesthesia was induced by intraperitoneal injection of pentobarbital (60 mg/kg; Sagatal; Rhone Merieux Ltd., Harlow, UK), and the depth of anesthesia was assessed by testing the withdrawal response to toe pinch and blink reflex. When there was no reaction to these tests, the surgical procedure was performed as described previously (Sahin and Rowland, 1998a). The bolus data were generated by single injections of nonequilibrated reference markers [normal red blood cells (RBCCs), $[^{125}$I]albumin, $[^{14}$C]sucrose, $[^{1}$H]urea, and $[^{1}$H]water] into the HA or PV and then, after an appropriate washout period, into the alternate vessel.

**Data Analysis.** All the effluent data were transformed to the fractional recovery rate data $f(t)$ using the following equation:

\[
f(t) = \frac{C(t) \cdot Q}{Dose}
\]

where $C(t)$ is the effluent concentration and $Q$ is the total perfusate flow (milliliters per second).

Arterial flow segregation was determined previously using a model based on the arterial and venous mean transit times and flow rates ($Q_{HA}$ and $Q_{PV}$), wherein the common and specific spaces are assumed to be arranged in parallel. Details of the model are given elsewhere (Sahin and Rowland, 1998b). The estimated arterial flow fractions ($f_1 = 0.83, f_2 = 0.17$) were fixed in the proposed single- and two parallel-component models before all curve fitting.
The fractional recovery rate output versus time data were analyzed using nonlinear regression analysis following numerical inversion of the appropriate Laplace equation (MULTI-FILT version 3.4, Yano et al., 1989) with a weighting scheme of 1/\(f(t)\), using the Damping Gauss algorithm, where \(f(t)\) is the predicted value of \(f(t)\) using the estimated parameters. The output data from the tubing alone were analyzed using eq. 7. When the one-compartment dispersion equation was fitted to the tubing data, the dispersion number \(D_{NT,NT}^{\text{in}}\) and the mean transit time (MTT,NT) associated with the nonhepatic region of the experimental system were found to be 0.04 and 2.3 s for the PV system and 0.04 and 2.5 s for the HA system, respectively. In all cases except urea and water, the transfer function of the nonhepatic region was defined by eq. 7 and incorporated to eqs. 3 and 4. In the case of urea and water, the time delay caused by the nonhepatic region was simply subtracted from the sampling time.

Regardless of the compound, all hepatic outflow profiles obtained following bolus PV input were described by the single component (common space) model (eq. 3), whereas the two-parallel component (common and specific space) model (eq. 4) was fitted to the hepatic effluent following arterial input. The common space parameters (e.g., \(D_{NC}^{\text{in}}\) and \(V_{NC}^{\text{in}}\)) were estimated from the PV output data, whereas the specific arterial space parameters (e.g., \(D_{NS,NC}^{\text{in}}\) and \(V_{NS,NC}^{\text{in}}\)) were estimated from the HA output data, by defining the common space parameters estimated from the PV effluent data, in the two-parallel component model. For all (noneliminated) reference markers (e.g., RBCs, albumin, sucrose, urea, and water), a one-compartment model (i.e., instantaneous radial distribution between the vascular and hepatic distributional spaces) was used. The goodness of fit was judged by visual observation, the distribution of residuals, and the coefficient of variation associated with the parameter estimates.

Simulations. Based on parameter estimates, simulations were performed to characterize the contribution in time of the common and specific space to the total hepatic output profile.

Statistical Analysis. All tabulated results were expressed as mean ± S.E.M. The results were compared by means of a Student’s paired or unpaired t test or analysis of variance to test the differences in \(D_{NC}^{\text{in}}\) and \(V_{NC}^{\text{in}}\) values of the reference markers estimated by applying the dispersion model. A p value of less than 0.05 was considered significant.

Results

The one-compartment single component (common space) and two-parallel component (common and specific space) models adequately described the outflow profiles obtained following bolus administration into the PV and HA, respectively. Representative fractional recovery rate outflow versus midterm profiles of RBCs, albumin, sucrose, urea, and water obtained following bolus administration into the PV are shown together with the line of the best fit by the single-component form of the dispersion model (Fig. 2A). The outflow profiles for the corresponding markers following arterial input are depicted in Fig. 2B. The contribution of common and specific spaces to the total outflow is illustrated in the simulation profiles (Fig. 3).

The parameters obtained by applying the single and two parallel component forms of the dispersion model are presented in Table 1. The common and specific space parameters of the proposed model were estimated with a high degree of precision (coefficient of variation of less than 10%), and the goodness of fit was improved when the weighting scheme of \(1/f(t)\) was used. The common space represented by far the larger portion of the total distributional space (common space + specific space) associated with each reference marker, with the specific arterial space constituting between 4 and 11%, with a mean value of 9% (Table 1). Even so, regardless of the reference marker (RBCs, albumin, sucrose, urea, and water), the estimated distributional volume (ml/g liver) following arterial administration was larger than the corresponding one following venous administration (\(p < 0.0001\)).

Although the dispersion number \(D_{N}^{\text{in}}\) related with the common space varied from 0.08 (RBCs) to 0.23 (water), those characterizing the specific space were very similar among the reference compounds with no significant difference between them (Table 1). For all except the extravascular markers (albumin and sucrose), the \(D_{N}^{\text{in}}\) values of the common space were significantly higher than those of the specific space (\(p < 0.001\)).

Discussion

In the development of the model, it was assumed that there is no specific portal space. Nor, unlike in the case of a specific arterial space, is there any suggestion or support for such. Theoretically, the presence of a specific portal space should be detected by predicted differences in the output profiles after PV input operating in the dual- and single-perfusion modes. In practice, we could discern no such difference between these perfusion modes, which could mean that either there is no specific portal space or it is too small to be detected under the experimental conditions. This was not the case for the HA-specific space (Sahin and Rowland, 1998b). Two models (serial and parallel models) have been developed to describe the connection between specific and common spaces (Field and Andrews, 1968; Sahin and Rowland, 1998b). The assumptions of both models are very similar made but they differ structurally. In the serial model, the specific spaces are serially connected to the common space, whereas in the parallel model these are in parallel with the common space. Although both models consider HA flow segregation, only the parallel model provides an estimate for the degree of the arterial flow segregation. It may be argued that neither model truly reflects reality. However, experiments on dual perfused livers revealed the presence of arteriovenous shunts, with some of the arterially delivered RBCs reaching the terminal hepatic venules via direct channels without traversing the sinusoidal bed (Sherman et al., 1996). Furthermore, existence of an isolated hepatic artery unaccompanied by a portal vein or a bile duct has been shown in various mammalian livers (Ekatasin, 2000), favoring the parallel model. Therefore, it was chosen in the present study to characterize the disposition kinetics of common and specific spaces.

The rate equations defining the axial dispersion model are second-order partial differential equations (e.g., eq.1) and require a set of boundary conditions to be solved by either analytical or numerical methods. We choose to use the mixed boundary conditions, which have been widely applied in the analysis of hepatic outflow data (Evans et al., 1991, 1993; Diaz-Garcia et al., 1992; Chou et al., 1993; Hussein et al., 1994). Although we could have chosen other boundary conditions, if the \(D_{N}^{\text{in}}\) value is less than around 0.2, as found in the current study irrespective of the marker and the space (common and specific), the solutions and estimated value of \(D_{N}^{\text{in}}\) are insensitive to the choice of the boundary conditions (VanGennuchten and Parker, 1984; Hisaka and Sugiyama, 1999). Furthermore, we investigated the impact of choice of boundary conditions on the parameter estimates by reanalyzing the frequency profiles of urea and water and found that the closed boundary model did not describe the outflow data better than the mixed boundary model (Sahin et al., 2005). Therefore, only the mixed boundary conditions are considered in the present study.

When analyzing the impulse response data, it is important to realize that the shape of the transit time distribution is determined not only by events within the liver but also by those occurring within the nonhepatic region of the experimental system (Goresky and Silverman, 1964; Luxon and Forker, 1982; Evans et al., 1991; Rowland and Evans, 1991). Apparatus dispersion is particularly important when modeling the outflow profiles of substances that have short mean transit times, such as red blood cells, albumin, and sucrose. Therefore, for these markers, the mean transit time and \(D_{N}^{\text{in}}\) of the nonhepatic...
FIG. 2. Linear plots of observed (●) fractional recovery rate outflow versus time profiles of RBCs, albumin, sucrose, urea, and water following bolus administration into the PV (A) and HA (B) of dual perfused liver preparation. The solid lines (—) represent the best fit lines by the one-compartment dispersion model.
region associated with each input were incorporated in the model. On the other hand, for urea and water with much longer transit times, distortion caused by the experimental system is less significant, so that the delay in the nonhepatic region can be accommodated simply by subtracting the lag time from the sampling time (Rowland and Evans, 1991).

Irrespective of the route of input, all the reference markers displayed monophasically declining outflow profiles following bolus administration into the PV and HA, consistent with the assumption of instantaneous radial distribution within the liver. Therefore, only the one-compartment form of the models (i.e., single component for PV, and two-parallel component for HA input) was applied to the fractional recovery rate outflow data. Although Weiss et al. (1997) criticized the one-compartment dispersion model for poor description of the tail part of the hepatic outflow curves of vascular indicators, and proposed a sum of two inverse Gaussian functions as an alternative, this was not found necessary. Both the common space (from the PV data) and specific arterial space (from the HA data) were successfully characterized, and their parameters were estimated with a high degree of precision (i.e., a coefficient of variation of less than 10%).

Albumin was studied in addition to sucrose as a marker of the extracellular space since many drugs are highly bound to this plasma protein, and its dispositional characteristics reflect those of albumin-bound drugs, which readily access the space of Disse and so bring albumin-bound drugs to the surface of the hepatocyte.

### Table 1

*Model-dependent analysis of the outflow profiles of reference markers obtained following bolus administration into the PV and HA (mean ± S.E.M., n = 5–25)*

<table>
<thead>
<tr>
<th>Marker</th>
<th>$D_{nc}$ (ml/g)</th>
<th>$V_{nc}$ (ml/g)</th>
<th>$D_{sa}$</th>
<th>$V_{sa}$ (ml/g)</th>
</tr>
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<tbody>
<tr>
<td>RBC</td>
<td>0.08 ± 0.01</td>
<td>0.166 ± 0.010</td>
<td>0.05 ± 0.01</td>
<td>0.010 ± 0.001</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.13 ± 0.01</td>
<td>0.214 ± 0.011</td>
<td>0.08 ± 0.02</td>
<td>0.024 ± 0.002</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.10 ± 0.01</td>
<td>0.222 ± 0.010</td>
<td>0.11 ± 0.02</td>
<td>0.025 ± 0.001</td>
</tr>
<tr>
<td>Urea</td>
<td>0.23 ± 0.03</td>
<td>0.609 ± 0.040</td>
<td>0.11 ± 0.01</td>
<td>0.059 ± 0.003</td>
</tr>
<tr>
<td>Water</td>
<td>0.23 ± 0.01</td>
<td>0.625 ± 0.034</td>
<td>0.12 ± 0.02</td>
<td>0.081 ± 0.005</td>
</tr>
</tbody>
</table>

*The common space parameters estimated from PV outflow data.
† Specific space parameters estimated from HA data by defining the common space parameters in the two-parallel component model.

![Fractional recovery rate outflow versus time profiles of RBCs, albumin, sucrose, urea, and water simulated by one-compartment dispersion model following arterial input. The solid (–), dashed (––), and dotted (..) lines represent the total, common, and specific space profiles, respectively.](image-url)

**Fig. 3.** Fractional recovery rate outflow versus time profiles of RBCs, albumin, sucrose, urea, and water simulated by one-compartment dispersion model following arterial input. The solid (–), dashed (––), and dotted (..) lines represent the total, common, and specific space profiles, respectively.
reference markers in the isolated perfused rat liver. Some are small, lying in the range 0.054 to 0.13 (Burczynski et al., 1996; Ueda et al., 1997), whereas others are much larger, within the range 0.2 to 0.5 (Robert et al., 1988; Evans et al., 1991). In the present study, DN estimates of both the common and specific arterial spaces were small (<0.25) and tended to be greater for the common (0.08–0.23) than specific (0.05–0.12) space. Similar DN estimates for all five reference markers in the specific space supports the idea that dispersion of these noneliminated compounds is due to a common feature, the microvasculature of this space. The magnitude of DN reflects the degree of axial heterogeneity occurring within the liver: the higher the DN value, the greater the degree of heterogeneity. The hepatic microvasculature comprises both large and microvessels (the sinusoids) with radial distribution into the interstitial and cellular spaces occurring only within the sinusoids. Therefore, the impact of any delay caused by the large vessels on the estimate of DN is greatest for compounds having the shortest transit times, that is, red blood cells and to a lesser extent the extracellular markers, albumin and sucrose. Evidence supporting this comes from several studies. One found that the estimate of the DN of bromosulphophthalein-glutathione and hippuric acid was 2- to 3-fold, respectively, when the lag time associated with the large vessels was taken into account rather than ignored (Tirona et al., 1998). Likewise, in perfusion studies investigating the use of catheter tubing of varying lengths, different DN estimates were observed for noneliminated reference indicators (RCBs, albumin, sucrose, and water) when the resulting lag time was ignored. However, a common estimate of DN (0.22) for all markers was obtained when a lag time (about 4 s) was included in the dispersion model (Schwab et al., 1998).

With regard to volumes of distribution, the dispersion model predicted very similar values to estimates based on statistical moment analysis (Sahin and Rowland, 2000a). For example, for the common space, VC (moment analysis versus dispersion model): 0.153 versus 0.166 ml/g for RBCs, 0.201 versus 0.214 ml/g for albumin, 0.216 versus 0.222 ml/g for sucrose, 0.598 versus 0.609 ml/g for urea, and 0.617 versus 0.625 ml/g for water. Similarly, observations following arterial administration for VC + Vm (moment analysis versus dispersion model): 0.173 versus 0.176 ml/g for RBCs, 0.221 versus 0.238 ml/g for albumin, 0.243 versus 0.247 ml/g for sucrose, 0.662 versus 0.668 ml/g for urea, and 0.690 versus 0.706 ml/g for water. The consistently smaller estimate for albumin rather than sucrose as a measure of the extracellular volume reflects the exclusion of large macromolecules from some of the space of Disse imposed by its polymeric gel-like matrix.

Irrespective of the aqueous space (e.g., vascular, extracellular, and intracellular) within the liver, the common space represents by far the largest portion (Table 1 and Fig. 3), with the specific space occupying only about 9% of the total space (common + specific space), in agreement with our previous estimate of 9 to 12% based on statistical moment analysis (Sahin and Rowland, 2000a).

The knowledge obtained from the existence of a specific HA water space and its flow fraction can be extended to make predictions about the fate of an eliminated substance after arterial administration. If the enzyme distribution responsible for elimination is the same as in the common space, the route of administration should have no effect on the disposition of a substance within the liver. In contrast, if there is no enzyme in the specific HA space, up to 17% of the HA dose will escape extraction, on the basis of the assumption that separation of the dose is a function of the flow. Recently, an elevated fractional hepatic recovery obtained for diazepam after HA than PV input (0.046 versus 0.019) suggests that the activity of the enzymes responsible for diazepam metabolism is less within the specific arterial than common space (Sahin and Rowland, 2000b). These findings have potential implication concerning the influence of route of administration on the hepatic extraction of compounds. After oral administration, compounds are delivered to the liver via the PV only and so will perfuse the common space with its higher enzymatic activity and extraction ratio. Although both the HA and PV convey the molecules to the liver after i.v. or parenteral administration, some will perfuse the specific arterial space, yielding a somewhat lower overall extraction ratio. The extreme situation arises with HA administration, which is occasionally used for the treatment of hepatic tumors, where a much higher fraction of the dose may escape into the systemic circulation than experienced with portally derived drug following oral administration.

References


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