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A Note on the Interpretation of Tracer Dispersion in the Liver

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The transit time distribution of intravascular markers and highly diffusible solutes is determined by the mixing process within the network of interconnected sinusoids. Based on the role of the relative dispersion or coefficient of variation (CV^2) of transit times as a measure of distribution dynamics (macromixing) various intrahepatic mixing processes are discussed, which are implied by current models of hepatic elimination. The opposite extremes of perfect micromixing and complete segregation are reflected by the dispersion model and the distributed parallel tube model, respectively. Assuming various capillary structures—including that of a fractal network—the dispersion models differ with regard to the predicted scaling behaviour of CV^2. The observed flow independence of CV^2 suggests that molecular diffusion and Taylor dispersion can be neglected but does not allow discrimination between mixing models.

Introduction

Multiple-indicator dilution technique has been the standard approach for estimating the distribution spaces of non-eliminated references such as labelled red cells, sucrose, and water (as vascular, extracellular, and cellular markers, respectively) (e.g. Goresky, 1963, 1983; Pang *et al.*, 1988). Application of this transient response technique is based on the identity of the impulse response (outflow curve $C(t)$) of the system with the density function of the transit time distribution (TTD) of molecules across the organ

$$f(t) = C(t)/\int_0^\infty C(t)\mathrm{d}t.$$

Then the moments of the TTD function

$$m_i = \int_0^\infty t^i f(t)\mathrm{d}t, \qquad (1)$$

define the expectations $E[T^i] = m_i$ of the random transit times T, i.e. the mean transit time $MTT = E[T]$ and the variance $VTT = E[T^2] - (E[T])^2$. Where:

$$MTT = m_1,\ VTT = m_2 - MTT^2. \qquad (2)$$

The model-independent parameter MTT is completely determined by the volume, V, accessible to the solute and the volumetric flow rate, Q:

$$MTT = V/Q. \qquad (3)$$

Although the outflow curve contains more information on the system than that given by MTT, there has been comparatively less interest in the interpretation and evaluation of parameters derived from higher curve moments, as the relative dispersion (CV^2) or normalized variance of transit time

$$CV^2 = \frac{VTT}{MTT^2}. \qquad (4)$$

In contrast to MTT the parameter CV^2 is not simply dependent on the distribution volume at steady state (eqn (3)) but on the dynamics of the distribution process (Weiss & Pang, 1992; Weiss, 1995); therefore

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CV^2 cannot be explained without a more detailed model of the liver. As in continuous chemical reactors (Dankwerts, 1953) intrahepatic mixing can be described in terms of two components, *macromixing* and *micromixing*. Macromixing determines the observed TTD; consequently, the parameter CV^2 provides one possible metric to quantify the degree of hepatic macromixing. A large variety of models of micromixing within the liver (mixing at a molecular level), however, can be consistent with any TTD. Thus the underlying micromixing process is not uniquely determined by the observed TTD: since in the case of linear kinetics the hepatic elimination of (highly extracted) drugs is dependent on macromixing alone (Dankwerts, 1953; Roberts *et al*., 1988) information on the latter process obtained by studies of the transient kinetics of intravascular markers can contribute to our understanding of hepatic pharmacokinetics. However, focusing on the transient case this note is not intended to discuss the validity of various elimination models at steady state (see e.g. Roberts & Rowland, 1985; Bass *et al*., 1987; Morgan & Smallwood, 1990). For example, the limiting cases of macromixing represented by the well-mixed and the plug-flow model are not considered. Although these models may be useful in predicting drug extraction at steady state they would imply completely unrealistic TTDs, i.e. single pass output profiles such as monoexponential and impulse functions, respectively. Based on the observed CV^2 values (or corresponding measures of dispersion) reported in the literature the purpose of this note is to discuss the structural implications of current models of intrahepatic distribution and elimination.

Liver Models

With regard to the assumed capillary structure there exist two opposite extremes among liver models:

M1: parallel, non-interacting sinusoids join at the venous terminus, i.e. flow is completely segregated within the liver and mixing occurs only at the venous side.
M2: network of interconnected sinusoids, which is regarded mathematically as a quasi-homogeneous medium, i.e. mixing occurs in the whole liver.

While the models may show the same macromixing behaviour (reflected by the observed relative dispersion, CV^2) the underlying micromixing processes are different. In so-called distributed models (M1) the dispersion characteristic, which is due to the velocity and path length variations within the ensemble of sinusoids, is incorporated into the model either *a priori* (Bass *et al*., 1978), assuming, for example, a Gaussian distribution (Forker & Luxon, 1978) or *a posteriori*, using the transit time distribution of red blood cells (Goresky, 1983).

The dispersion model (M2) is based on the convection–dispersion equation, one of the most famous equations in chemical engineering. This concept was introduced into pharmacokinetics by Roberts & Rowland (1985, 1986), and has also been previously applied to the problem of tracer dispersion in the kidney (Perl & Chinard, 1967). [As a matter of historic interest the first application of this concept to indicator dilution curves goes back to Sheppard & Savage (1951).] The convection–dispersion equation (CDE) represents a macroscopic description of tracer dispersion

$$\frac{\partial C}{\partial t} + U\frac{\partial C}{\partial x} - D\frac{\partial^2 C}{\partial x^2} = 0, \tag{5}$$

where U is the average flow velocity assumed to be in the x direction and D is the dispersion coefficient. Often the CDE is represented in dimensionless form by substituting D with the so-called dispersion number:

$$D_N = \frac{D}{UL}, \tag{6}$$

where L is the length of the system that accounts for the mean transit time, thus $L = MTT\ U$. Note that $U = Q/A$, where A is the cross-sectional area for hepatic blood flow, Q. The fact that only macroscopic averaged variables are used in the CDE implies that the heterogeneous sponge-like medium is regarded as being *effectively homogeneous at a macroscopic scale*. At the microscopic level, flow is assumed as random in space and time. This concept implies that the magnitude of the dispersion number D_N is completely determined by the process of intrahepatic mixing (e.g. Roberts & Rowland, 1986; Bass *et al*., 1987; Roberts *et al*., 1988; Rowland & Evans, 1991).

Geometrical Dispersion

Geometrical dispersion, which depends solely on the morphology of the capillary network has been the idea implicit in the application of the CDE to the liver (M2): for high velocities D is controlled by mechanical mixing of blood at the junctions of branching and interconnecting sinusoids leading to different flow paths and velocities for individual molecules, which causes a distribution of transit times. In view of the random nature of this network of sinusoids stochastic models of dispersion appear

appropriate. Mathematically such a purely convective dispersion process can be regarded as analogous to tracer dispersion in heterogeneous porous media. This dispersion process is due to the complex trajectory of individual particles in the geometrical disordered medium at larger flow rates (e.g. Scheidegger, 1974; Koplik *et al*., 1987). Here I will give a simple interpretation of one of the results of this theory, which appears most interesting in the present context. Consider a network of branches and junction nodes, where complete mixing occurs and in which the fluid velocity is changed in direction and magnitude. Defining a characteristic microstructural length scale, l, as the path length after which the velocity of a tracer molecule becomes decorrelated from its original value, then l is the typical length between junctions. Thus, the random network can be approximated by a chain of well-mixed compartments connected by parallel branches of length l, and the dispersion process may be regarded as a random walk on a line with step of magnitude l and with a frequency U/l. Since the typical time for one step is of the order of $\tau = l/U$, one can conclude that dispersion with respect to average flow is characterized by $D = l^2/\tau$ or

$$D = Ul \tag{7}$$

and the dispersion number is completely determined by the length ratio

$$D_N = \frac{l}{L}. \tag{8}$$

In order to estimate D_N from the normalized variance CV^2 of TTD, the CDE has to be solved for particular initial and boundary conditions. In general, the identity of impulse response of the system and the TTD density is lost due to the diffusion-like phenomena at the boundaries (Nauman, 1981). However, implicit in the use of the CDE is macroscopic homogeneity

$$l \ll L, \tag{9}$$

i.e. the liver is conceptualized as being continuous with a microscopic scale l, which is small with respect to the macroscopic (global) scale L of the volume V. (It is an open question how the reference length L should be defined.) In other words, MTT has to be "much greater than any microscopic time τ in the system, so that the tracer is likely to fully sample the microscopic structure" (Koplik *et al*., 1987). This simplification, which is fundamental for the application of eqn (5), represents a general problem of transport in porous media (Adler, 1992). Consequently, the condition

$$D_N \ll 1, \tag{10}$$

is implicitly adopted in applying the dispersion model to the liver. The boundary conditions are unimportant for low D_N numbers (Bass *et al*., 1987) since the dispersion effects at the ends can then be neglected, and all differences between open and closed systems vanish. [They have the same TTD when the time spent outside the boundaries is excluded (Nauman, 1981).] Roberts *et al*. (1988), for example, found no difference between the model curves based on the conventional "closed-closed" and the "mixed" boundary conditions with regard to the goodness-of-fit of experimental data. Using the appropriate initial condition (see Hsu & Dranhoff, 1986) one obtains the following TTD density function from eqn (5):

$$f(t) = \sqrt{\frac{MTT}{2\pi CV^2 t^3}} \exp\left[-\frac{(t - MTT)^2}{2CV^2 MTT t} \right]. \tag{11}$$

Equation (11) represents the inverse Gaussian distribution; which has been introduced as first passage time distribution of intravascular indicators or solutes with flow-limited distribution (Sheppard, 1962; Norwich & Zelin, 1970; Weiss, 1982; Roberts & Rowland, 1986). The dimensionless variance of this TTD is $CV^2 = 2\ D_N$ and eqn (8) yields $CV^2 = 2\ l/L$.

Gaussian Convection

If the alternative standpoint is taken and eqn (11) is interpreted in terms of the distributed (parallel tube) model (M1) the resulting TTD is not governed by the CDE despite the fact that the outflow curves are of the same shape, i.e. that the TTDs are formally identical. Consequently, CV^2 is not related to a dispersion number, i.e. it is only a measure of the spread of the TTD reflecting the spread of the velocity and/or path length distribution. (Each blood element has its own constant velocity independently of the other blood elements.) One possible interpretation that is consistent with observed TTDs (eqn (11)) is that of a Gaussian velocity distribution, also known as a Gaussian-convective model (Levenspiel & Fitzgerald, 1983), where CV^2 is, by definition, independent of the length of the system L. Thus, there is a difference in the scaling behaviour of CV^2 with regard to the reference length L: $CV^2 =$ constant for the distributed model (M1), while the dispersion model (M2) predicts $CV^2 \propto L^{-1}$.

Interpretation of Experimental Data

It is well recognized that available experimental data obtained in the isolated perfused rat liver point to the fact that the observed dispersion number of $D_N \approx 0.3$ is solely dependent on the anatomy of the liver, both for intravascular indicators [one-compartment dispersion model, eqns (5) and (11)], discussed above, and for solutes with permeability limited distribution evaluated using the two-compartment dispersion model (Rowland & Evans, 1991; Diaz-Garzia *et al*., 1992; Hussein *et al*., 1994). Interestingly, the relative dispersion CV^2 (and consequently D_N) of Evans Blue and water (Roberts *et al*., 1990), as well as the dispersion number estimated for cefixime using the two-compartment dispersion model (Yano *et al*., 1991) were *independent of flow rate*. Unfortunately, this result does not allow a discrimination between the dispersion and the distributed model, since both the geometrical dispersion [cf. eqn (8)] and the dispersion due to Gaussian convection are flow independent. However, based on these results one can infer that the contributions of both molecular diffusion (D = constant) and Taylor dispersion due to laminar flow in tubes ($D \propto Q^2$) can be neglected in the observed flow range; since this would imply $CV^2 \propto Q^{-1}$ and $CV^2 \propto Q$ respectively, for intravascular indicators.

Adopting the dispersion model one is faced with the dilemma that—at least from a theoretical point of view—the observed value of $D_N \approx 0.3$ violates the underlying condition (eqn (10)). An alternative hybrid model of hepatic elimination was developed by Bass *et al*. (1987) to describe the influence of mixing on drug elimination at steady state. They assumed that $N-1$ mixing sites are located between N identical subsystems arranged in series, and that the individual subsystems act as conventional segregated distributed models. It appears interesting that the N values of 5.8 and 5.0 estimated by Bass *et al*. (1987) from hepatic extraction data of taurochlorate and colloid, respectively, are not much different from the result of $N = L/l = 3.3$ obtained from $CV^2 = 0.6$ assuming geometrical dispersion. It appears questionable, however, whether the hybrid model is useful for explaining the relative dispersion of non-eliminated solutes in the transient state since it reflects a functional rather than an anatomical structure. For example, the results of Bass *et al*. (1987) obtained from extraction data may also be influenced by the heterogeneity of intrahepatic enzyme distribution.

Scaling Behaviour

As discussed above there are pros and cons with regard to models M2 *and* M1. However, the question is certainly not whether the hepatic TTD is due to *perfect micromixing* (dispersion model) *or complete segregation* (distributed model). Although existing structural evidence suggests that there is no complete segregation, one may ask whether the existence of interconnecting sinusoids (Koo *et al*., 1975) or observations such as "sinusoids are organized into dense anastomosing capillary masses" (Kardon & Kessel, 1980) provide a sufficient basis for the application of the dispersion model. A number of about two effective mixing sites, i.e. a ratio l/L of about 0.3, however, appears not consistent with the underlying assumption of a quasi-homogenous medium. Consequently, one may conjecture that in the liver the real mixing behaviour lies somewhere between the opposite extremes. Following an idea of Levenspiel & Fitzgerald (1983) this intermediate situation could be characterized by the scaling behaviour of CV^2 according to a power function of L:

$$CV^2 \propto L^{-d} \qquad 0 \le d \le 1, \tag{12}$$

where the parameter d characterizes the position between the two micromixing limits represented by M1 and M2, namely complete segregation ($d = 0$) and perfect micromixing ($d = 1$).

Another hypothesis to explain the high degree of mixing in microvascular networks that has been put forward is based on the concept of space-filling networks as the morphological substrate of turbulence in the system (Sernetz *et al*., 1985; Bassingthwaighte, 1988), where a change from laminar to turbulent flow is assumed to be caused by the fractal vascular branching. Interestingly, adopting the scaling law for the dispersion coefficient predicted by the theory of diffusion in *fractal networks* (Adler, 1992):

$$D \propto L^{2-d_w}, \tag{13}$$

the relative dispersion would also scale according to eqn (12) but with $d > 1$ instead of $d \le 1$:

$$CV^2 \propto L^{-d} \text{ and } 1 \le d = d_w - 1. \tag{14}$$

Modelling the fractal network using a Sierspinski gasket, for example, leads to $d_w = 2.32$ (Adler, 1992) while for an Euclidian medium ($d_w = 2$) one again obtains the limiting case of perfect micromixing ($d = 1$). It should be noted, however, that in contrast to other organs (Bassingthwaighte, 1988) no fractal model is available for the hepatic microvasculature.

Furthermore, although the results appear interesting from a theoretical point of view, the practical usefulness of eqns (12) and (14) remains open as long as the anatomical correlates of the parameters L and l are unknown.

Concluding Remarks

It should be pointed out that the hepatic extraction ratios are independent of micromixing if drug elimination (e.g. steady state extraction) is dose independent (linear or first order kinetics) (Dankwerts, 1953). In contrast, the model of the intrahepatic mixing process may play a role if nonlinear kinetics are operative (Bass *et al*., 1987; Roberts *et al*., 1988). This has been demonstrated for propranolol and galactose in a simulation study assuming Michaelis–Menten kinetics (Roberts *et al*., 1989). Thus, the characterization of the intrahepatic mixing process is not only of interest for a better understanding of the functional structure of the liver microvasculature; it is also a prerequisite for the modelling of oral availability and the disposition of compounds undergoing saturable kinetics. Although there appears to be no method at hand for checking intrahepatic micromixing, one should be aware of the fact that different mixing concepts are implicitly assumed in liver modelling.

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