Consideration on Moments of Outflow Profile in Liver Perfusion System with Change in Perfusate Flow Rate Using Oxacillin as Model Drug

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The effect of perfusate flow rate on hepatic structure and hepatic uptake kinetics was investigated using oxacillin as a model drug and bovine serum albumin (BSA) as a reference substance in the liver perfusion system from the standpoint of a dispersion model and moment characteristics. The estimated recovery ratio \( F_{\text{H}} \) of oxacillin was about 40% which was independent of the change in perfusate flow rate. The mean transit time \( (t_{\text{H}}) \) of oxacillin decreased with an increase in flow rate, while the relative variance \( (\sigma^2/t_{\text{H}}^2) \) of oxacillin was independent of the flow rate. The \( t_{\text{H}} \) of BSA decreased with an increase in the flow rate to the same extent as that of oxacillin, while \( \sigma^2/t_{\text{H}}^2 \) of BSA was independent of flow rate. When the dispersion model is adopted as a model system to analyze hepatic perfusion data following the pulse input, the moment characteristics \( (F_{\text{H}}, t_{\text{H}} \) and \( \sigma^2/t_{\text{H}}^2 \) \) are given in complicated equations. It is demonstrated by the present investigation that these moment equations can be extensively simplified for a drug with a medium extraction ratio \( (F_{\text{H}} > 50\%) \), i.e., \( F_{\text{H}} \) is independent of the distribution, both \( F_{\text{H}} \) and \( t_{\text{H}} \) are independent of the dispersion process in the hepatic blood space, and both \( F_{\text{H}} \) and \( \sigma^2/t_{\text{H}}^2 \) are independent of the elimination. Thus, it is shown that \( F_{\text{H}} \) and \( t_{\text{H}} \) are exactly the indices of elimination and distribution, respectively, and \( \sigma^2/t_{\text{H}}^2 \) is the index of dispersion in the blood space plus nonequilibrium in the hepatic distribution.

Key words: rat liver perfusion; blood flow rate; dispersion model; MULTI(FILT); BSA; oxacillin

Hepatic blood flow rate is an important factor which characterizes local drug disposition in the liver. The hepatic blood flow rate is not constant and often fluctuates dependent on body condition.\(^1\) Since changes in hepatic function by blood flow rate can affect the global disposition of a drug in the body, it is important to understand the relationship between the blood flow rate and the local disposition in the liver. Perfusion experiments have been used to estimate local hepatic disposition. The hepatic clearance of drugs has been well examined under a steady state condition, and the clearance was often affected by the hepatic blood flow rate.\(^2\) Curve fitting based on dispersion models was adopted to precisely analyze outflow profiles of drugs in the liver perfusion system following the pulse input of a drug.\(^7\) The dispersion models offer tools to separately estimate the degree of drug mixing in the perfusate, the volume of blood space (the Disse space plus the sinusoidal space), and the extent of drug distribution and elimination processes from the perfusate into liver tissues. The effects of perfusate flow rate on hepatic disposition were investigated using cefixime\(^9\) and salicylic acid\(^10\) based on dispersion models. Both reports demonstrated that the dispersion number \( (D_{\text{H}}) \), which is an index of eddy mixing in the blood space, was independent of the perfusate flow rate. The parameters in dispersion models were correlated to the moment characteristics, such as the recovery ratio \( (F_{\text{H}}) \), the mean hepatic transit time \( (t_{\text{H}}) \), and the relative variance of the transit time \( (\sigma^2/t_{\text{H}}^2) \) in the relationship between the Laplace transform and moments.\(^7\) However, the derived equations are too complicated to conjecture the hepatic local disposition from the moment values.

The purpose of the present investigation is to evaluate the effect of perfusate flow rate on local hepatic disposition, using oxacillin as a model drug with medium hepatic clearance \( (F_{\text{H}} > 50\%) \) and bovine serum albumin (BSA) as a marker of blood space in the perfusion system following pulse input into the portal vein. It is demonstrated from the present result that the equations of \( F_{\text{H}} \), \( t_{\text{H}} \) and \( \sigma^2/t_{\text{H}}^2 \) can be extensively simplified for a drug with a medium hepatic extraction ratio, irrespective of the properties of the drug.

MATERIALS AND METHODS

Animal Experiment Male Wistar rats weighing from 191 to 230 g were anesthetized with pentobarbital (NEMBUTAL®, 50 mg/kg i.p.) and their livers were perfused in situ according to the Mortimore perfusion method.\(^2\) The perfusate (Kreb’s-Ringer bicarbonate buffer containing 10 mm glucose and maintained at 37°C by a water bath) was delivered into the liver through a cannula (1.67 mm o.d.) into a portal vein at a certain flow rate by a peristaltic pump (RP-N3, Furuie Scientific Co., Ltd., Tokyo, Japan). The perfuse flow rates were adjusted to 10.0, 15.0, 20.0 and 25.0 ml/min using oxacillin as a model drug, whereas flow rates of 15.0 and 25.0 ml/min were used with BSA as a reference substance. BSA and red blood cells were not dissolved in the perfusate to exclude the influence of these substances. Each group at single flow rate consisted of four rats \( (n=4) \). 0.1 ml of oxacillin solution (5 mg/ml) in the perfusate buffer or 0.1 ml of the solution of 4% BSA labeled with Evans Blue (39.8 mg/ml BSA and 3.85 mg/ml Evans Blue) was injected into the liver through the portal vein cannula using a HPLC ceramic injector (SVI-6U7, Sanuki Industry Co., Ltd., Tokyo, Japan). Outflow samples were collected at intervals of about 1 s for 20 up to 60 s, depending on the flow rate from a cannula inserted into the thoracic vena cava inferior. The exact sampling time was calculated from the eluted volume of each outflow sample at a constant flow rate. The viability of liver was monitored by the bile flow volume for several minutes throughout the experiments; that is, the perfusion data were not adopted.

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when the bile flow rate was less than 4 μg/min. The wet
livers were 8.97 ± 0.67 g. All perfusion experiments were
finished within 20 min from the start of the abdomen
incision of rats.

Chemicals Oxacillin (Staphicillin V) and bovine serum
albumin (fatty acid-free; fraction V) were obtained from
Sigma Chemical Co. (St. Louis, U.S.A.). Evans Blue was
purchased from Wako Pure Chemical Industries, Ltd.
(Osaka, Japan). All of the reagents for the Krebs–Ringer
bicarbonate buffer and for the mobile phase of HPLC
were of analytical grade.

Analytical Procedure In the case of BSA, Evans Blue
that was used as a labeling reagent for BSA was measured
by a spectrophotometry (UV-VIS, Shimadzu) at 620 nm.
Because of the high affinity of Evans Blue to BSA, its free
fraction was negligible.13) The blank perfusate just eluted
from the liver was spiked with various amounts of Evans
Blue, and the resulting solutions were used as the standard
solution in each experiment. The calibration lines were
calculated by a linear least squares method. The correlation
coefficient of the calibration line for BSA was more than
0.999 over the experimental concentration range (4—100
μg/ml).

In the case of oxacillin, a high-performance liquid
chromatograph (LC-3A, Shimadzu Co., Kyoto, Japan)
equipped with a UV-detector (SPD-2A, Shimadzu) and
an integrated data analyzer (Chromatapack C-R6A,
Shimadzu) was used. A stationary phase was Chemosorb
5-ODS-H (150 mm 4.6 mm i.d.; Chemco Co., Osaka,
Japan), and a mobile phase at a flow rate 1.0 ml/min was
acetate buffer-methanol solution (1:1, v/v, pH = 5.2).
The detection wavelength was 220 nm. The column temp-
perature was set at ambient temperature. A 15 μl portion of
each outflow sample was injected. The correlation
coefficient of the calibration line for oxacillin was more
than 0.999 over the experimental concentration range
(3—200 μg/ml).

In order to determine void volume (catheter volume
plus injector volume) and void time, the inflow cannula
was directly connected to the outflow tube and the outflow
profile of Evans Blue was measured after the bolus
injection at varied flow rates. The void volume estimated
by transit time × flow rate was 0.3 ml which was
independent of the perfusate flow rate. Before the curve
fitting, the void time (= void volume/flow rate) was
subtracted from the obtained sampling time data. The
variance of catheter transit time at the lowest flow rate
was 3.41 ± 0.94 s² (about 6% of total variance) which was
the maximum in the present perfusion experiment.
The broadening of the injected sample in the injector loop
and catheter was assumed to be negligible in analyzing the
outflow data.

Numerical Analysis All the drug disposition processes
of oxacillin and BSA were assumed to be linear. The
outflow time profiles of oxacillin and BSA after the bolus
input were evaluated in one- and two-compartment
dispersion models. When a drug is injected into the liver
as a pulse input, the image equations for one-compartment
and two-compartment dispersion models with central
elimination are given by Eqs. 1 and 2, respectively.7—9)

\[
\tilde{C}_s(t) = \frac{M}{Q} \exp \left[ \frac{QV_b}{2D_c} \left( 1 - \frac{4D_c}{Q^2} \left( k_c + (1 + k')\alpha \right) \right) \right]
\]

\[
\tilde{C}_s(t) = \frac{M}{Q} \exp \left[ \frac{QV_b}{2D_c} \left( 1 - \frac{4D_c}{Q^2} \left( s + k_{12} + k_{21} - \frac{k_{12} k_{21}}{s + k_{12}} \right) \right) \right]
\]

where \( M \) is the amount of dose injected, \( Q \) (ml/min) is
the flow rate of perfusate, \( D_c \) (ml²/min) (= \( D_c e^{A_1^2} \))
is the corrected dispersion coefficient, \( D_c \) is the effective
dispersion coefficient, \( A \) is the cross-sectional area of blood
space, \( V_b (ml) \) is the volume of hepatic blood space which
is the sum of the volume of the sinusoid and the Disse
space, \( k_{12} \) and \( k_{21} \) are the forward and backward transfer
rate constants, respectively, and \( k_e \) is the irreversible
transfer rate constant from the perfusate mainly into the
hepatic tissues. The ratio \( k' = (k_{12}/k_{21}) \) is the partition
ratio, which is an index of the extent of drug distribution
between the perfusate and hepatic tissues. Equations 1
and 2 have been rearranged from the original equations.7) It
is demonstrated that the dispersion model with peripheral
elimination is kinetically equivalent to that of the central
elimination model. In the central elimination model,
eliminination means the irreversible movement of a drug
into hepatic tissues and distribution means the reversible
movement between the blood space and hepatic tissues.
The hepatic recovery ratio \( (F_R) \), the mean transit time \( (t_R) \) and the relative variance (\( \sigma^2/\tilde{F}_R^2 \)) of the two-compartment
dispersion model are given by

\[
F_R = \exp \left[ \frac{QV_b}{2D_c} \left( 1 - \frac{4D_c k_c}{Q^2} \right) \right]
\]

\[
t_R = V_b (1 + k') \frac{Q}{Q^2}
\]

\[
\sigma^2/\tilde{F}_R^2 = \frac{2D_c}{QV_b} \left( 1 + \frac{4D_c k_c}{Q^2} + \frac{k_e Q}{k_{21}} + \frac{2k_e Q}{V_b (1 + k')} \right) \frac{1 + 4D_c k_c}{Q^2}
\]

Equations 3 and 4 are valid for a one-compartment
dispersion model. Equation 5 is reduced to that of a
one-compartment model by neglecting the second term
in Eq. 5 \( (k_{21} \rightarrow \infty) \). Therefore, \( k_{21} \) is an index of non-
equilibrium in the distribution between blood space and
hepatic tissues.

The dispersion number \( (D_N) \) and the efficiency number
\( (R_N) \) are calculated by Eqs. 6 and 7, respectively.

\[
D_N = D_c Q V_b
\]

\[
R_N = k_e V_b / Q
\]

The curve fittings of Eqs. 1 and 2 to the outflow profiles
of cefxime and BSA from the liver were carried out by
means of MULTI (FILT)14,15 in the mainframe computer
(M-1800/30) in the Kyoto University Data Processing
Center. In the curve-fitting to BSA data, the elimination
rate constant, \( k_e \), was assumed to be zero.16) The optimum
dispersion model was tested by Akaike’s information
criterion (AIC).17) The one- or two-compartment model
which had a smaller AIC value was assumed to be better.
The moment values of \( F_R \), \( t_R \) and \( \sigma^2/\tilde{F}_R^2 \) were calculated
both by Eqs. 3—5 following the curve fitting and by a
trapezoidal integration into infinite time.19)
RESULTS

It was known, following the analysis of AIC, that all outflow-time profiles of BSA were well approximated by the two-compartment model. Only one outflow profile of oxacillin at 25 ml/min was well represented by the one-compartment model, while the other output time profiles of oxacillin were represented by the two-compartment model. Therefore, the parameters for oxacillin and BSA in the two-compartment dispersion model are listed in Tables 1 and 2, respectively. The moment characteristics by Eqs. 3–5 and by trapezoidal integration are also included in Tables 1 and 2. The moments calculated from the kinetic parameters by the curve fittings were almost the same as those by the trapezoidal integration to infinite time.

Concerning oxacillin kinetics, $D_N(=D_AA^2)$ increased with an increase in the flow rate of the perfusate, while $D_N$ was independent of the flow rate. $V_N$ increased with an increase in the flow rate. Therefore, the increase in $D_N$ is explained by an increase in the cross-sectional area of blood space. $k_s$ was independent of the flow rate, while $k'$ decreased with an increase in flow rate. $k_{21}$ was almost independent of the flow rate. The estimated recovery ratio ($F_H$), ranging from 40% to 50% of the dose, was independent of the flow rate. The mean transit time ($t_M$) decreased with an increase in the flow rate, whereas the relative variance ($\sigma^2/t_M$) was not affected by the perfusate rate. The dispersion number ($D_N$), which is an index for eddy mixing in the perfusate, and the efficiency number ($R_N$) were almost independent of the flow rate.

Concerning BSA kinetics, all parameters except $k_s$ in the dispersion model changed with the flow rate in the same manner as those of oxacillin. $t_M$ decreased with an increase in the flow rate, while $\sigma^2/t_M$ was almost independent of the flow rate. $D_N$ (or $D_N$) and $V_N$ are naturally expected to be independent of the properties of drugs but dependent on the structure of the blood space in the liver. Actually, the differences in $D_N$ (or $D_N$) and $V_N$ were insignificant between oxacillin and BSA at 15 and 25 ml/min (ANOVA, 5% level). The $V_N$ values of oxacillin and BSA were about 20% of liver weight at 15 ml/min flow rate, which is slightly greater than 15.5% in the previous reports.\(^\text{16}\)

DISCUSSION

In order to comprehend local hepatic disposition based on moment values, it is helpful to simplify Eqs. 3, 4 and 5. It is known in these equations that a common factor, which we temporally call $R$, is noticed.

$$ R = \sqrt{1 + 4D_Nk_s/Q^2} = \sqrt{1 + 4D_NR_N} \tag{8} $$

In Eq. 8, $R$ includes $D_N$ and $R_N$ which are the most basic indices in the dispersion model. Figure 1 shows $R$ versus the flow rate of perfusate. The solid line shows $R$ of oxacillin calculated from the kinetic parameters in Table 1. The $F_H$ values of oxacillin are about 40% at any flow rate. $R$ of oxacillin took 1.04 (+0.005) which was very close to unity. The dotted lines show the predicted $R$ using Eq. 8 under the condition that $D_N$ values are fixed to those of oxacillin at each flow rate and $R_N$ values are increased. Consequently, $F_H$ decreases from 40% down to 5%. Even in the case, $F_H=95\%$, $R$ was still close to unity (1.13±0.01). It has been shown in previous studies, using

<table>
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<tr>
<th>Flow rate (ml/min)</th>
<th>10.0</th>
<th>15.0</th>
<th>20.0</th>
<th>25.0</th>
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<tbody>
<tr>
<td>Liver weight (g)</td>
<td>9.65</td>
<td>8.05</td>
<td>9.91</td>
<td>8.92</td>
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<tr>
<td>$D_N$ (ml²/min)</td>
<td>0.327</td>
<td>0.628</td>
<td>1.01</td>
<td>1.69</td>
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<tr>
<td>$V_N$ (ml)</td>
<td>1.73</td>
<td>1.88</td>
<td>2.41</td>
<td>3.03</td>
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<tr>
<td>$k_{12}$ (min⁻¹)</td>
<td>5.13</td>
<td>3.33</td>
<td>4.36</td>
<td>2.47</td>
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<tr>
<td>$k_{21}$ (min⁻¹)</td>
<td>34.2</td>
<td>29.0</td>
<td>41.9</td>
<td>32.9</td>
</tr>
<tr>
<td>$k_s$ (min⁻¹)</td>
<td>5.23</td>
<td>7.40</td>
<td>6.33</td>
<td>7.87</td>
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<tr>
<td>$k'$ (= $k_{12}/k_{21}$)</td>
<td>0.153</td>
<td>0.114</td>
<td>0.103</td>
<td>0.0795</td>
</tr>
<tr>
<td>$F_H$ (%)(^a)</td>
<td>41.5</td>
<td>40.8</td>
<td>47.3</td>
<td>39.3</td>
</tr>
<tr>
<td>$t_M$ (s)</td>
<td>11.5</td>
<td>8.04</td>
<td>7.72</td>
<td>7.54</td>
</tr>
<tr>
<td>$\sigma^2/t_M^a$</td>
<td>0.0787</td>
<td>0.0969</td>
<td>0.0818</td>
<td>0.0911</td>
</tr>
<tr>
<td>$F_N$ (%)(^b)</td>
<td>42.6</td>
<td>41.2</td>
<td>47.8</td>
<td>40.5</td>
</tr>
<tr>
<td>$t_M$ (s)</td>
<td>11.9</td>
<td>8.01</td>
<td>7.70</td>
<td>7.41</td>
</tr>
<tr>
<td>$\sigma^2/t_M^b$</td>
<td>0.116</td>
<td>0.0998</td>
<td>0.0859</td>
<td>0.0862</td>
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</tbody>
</table>

Data are shown as mean (±S.D.); n = 4. \(^a\) Calculated by Eqs. 3–5. \(^b\) Calculated by trapezoidal integration into infinite time.

<table>
<thead>
<tr>
<th>Flow rate (ml/min)</th>
<th>15.0</th>
<th>25.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight (g)</td>
<td>8.73</td>
<td>8.99</td>
</tr>
<tr>
<td>$D_N$ (ml²/min)</td>
<td>0.788</td>
<td>2.45</td>
</tr>
<tr>
<td>$V_N$ (ml)</td>
<td>1.76</td>
<td>2.88</td>
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<td>$k_{12}$ (min⁻¹)</td>
<td>4.20</td>
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<tr>
<td>$k_{21}$ (min⁻¹)</td>
<td>14.2</td>
<td>10.9</td>
</tr>
<tr>
<td>$k_s$ (min⁻¹)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k'$ (= $k_{12}/k_{21}$)</td>
<td>0.293</td>
<td>0.237</td>
</tr>
<tr>
<td>$F_H$ (%)(^a)</td>
<td>99.3</td>
<td>98.6</td>
</tr>
<tr>
<td>$t_M$ (s)</td>
<td>9.90</td>
<td>8.18</td>
</tr>
<tr>
<td>$\sigma^2/t_M^a$</td>
<td>0.292</td>
<td>0.278</td>
</tr>
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</table>

Data are shown as mean (±S.D.); n = 4. \(^a\) Calculated by Eqs. 3–5. \(^b\) Calculated by trapezoidal integration into infinite time.
mainly β-lactam antibiotics, that $D_N$ was independent of the properties of these drugs, with values less than 0.1 (7–9, 11). It was shown in the present investigation that the $D_N$ values of oxacillin (model drug) almost coincided with those of BSA (marker in the blood space), and both $D_N$ values were independent of the flow rate as shown in Tables 1 and 2. Thus, $R$ for a drug with an intermediate extraction ratio is expected to be close to unity, irrespective of the properties of the drug.

When $4D_N k_w/Q^2 (= 4D_N R_0)$ is sufficiently smaller than unity, Eqs. 3, 4 and 5 can be extensively reduced to Eqs. 9, 10 and 11, respectively.

$$F_H = \exp \left( -\frac{k_w V_B}{Q} \right)$$

(9)

$$I_{th} = \frac{V_B (1 + k)}{Q}$$

(10)

$$\sigma^2 / I_{th}^2 = I_{th} = \frac{2D_N}{QV_B} + \frac{2Qk}{k_wV_B(1+k)^2}$$

(11)

Equation 9 is derived from the Taylor’s expansion with respect to $4D_N k_w/Q^2$ in Eq. 3. Equation 10 is equal to the theoretical equation for the retention time and Eq. 11 corresponds to that for the height equivalent to a theoretical plate (HETP) in the chromatographic field. 29) It is known in Eq. 9 that $F_H$ is independent of both the eddy mixing and the distribution between the perfusate and the liver tissues and it coincides with that of the parallel tube model. 5) In the case of drugs with an extremely high clearance, $F_H$ is predicted to increase with an increase in the flow rate under the steady-state condition. 3, 6, 10) In the case of oxacillin with intermediate clearance, $F_H$ was independent of the flow rate, which is explained that $k_wV_B$ in Eq. 9 proportionally increased with an increase in $Q$. Actually, the $V_b$ values of both oxacillin and BSA increased twice with an increase in the flow rate from 15 to 25 ml/min. This explanation agrees with reports that blood volume increased with the flow rate. 21) It is also found from Eq. 10 that the mean transit time $(t_{th})$ is independent of both the elimination of a drug and the eddy mixing, and hence it can be an index of the extent of drug distribution. The $t_{th}$ of oxacillin was very close to that of BSA, which is simply explained that oxacillin and BSA have almost the same small $k_w$ values in Eq. 10. In Eq. 11, the relative variance $(\sigma^2 / I_{th}^2)$ consists of two terms that are independent of the drug elimination. The first term is related to eddy mixing in the blood space of the liver and the second to the nonequilibrium distribution between the perfusion and the liver tissues. When the liver system can be expressed by the one-compartment dispersion model, Eq. 11 is reduced to Eq. 12 by neglecting the second term.

$$\sigma^2 / I_{th}^2 = 2D_N$$

(12)

$D_N$ is almost independent of the perfusate flow rate for a drug with a medium extraction ratio, and is recorded at about 0.4 as the maximum in the two-compartment dispersion model. 22, 23) Therefore, when $\sigma^2 / I_{th}^2$ is greater than 0.8, there is a possibility that the nonequilibrium distribution is not negligible. In the present experiment, $\sigma^2 / I_{th}^2$ of oxacillin and BSA were small, and the $k_w$ values were considerably large as shown in Tables 1 and 2. Thus, the output time profiles of oxacillin and BSA are close to those of one-compartment dispersion model, although AIC estimation suggests the two-compartment model is a better model.

In conclusion, it is shown that, for a drug with intermediate extraction ratio, $I_{th}$ is an index for the extent of elimination, $t_{th}$ for the extent of distribution, and $\sigma^2 / I_{th}^2$ for eddy mixing in the blood space plus nonequilibrium in the distribution. These indices offer a simple means to understand local hepatic disposition based on the moment values.

REFERENCES