Verapamil is a calcium-channel antagonist that can reduce the strength and rate of contraction of cardiac muscle (Cohen et al., 1987; Satoh et al., 1979; Raschack, 1976) and the tone of vascular smooth muscle (Nawrath and Raschack, 1987; Golenhofen and Lammel, 1972). It has been used clinically to treat hypertension, myocardial ischemia and tachyarrhythmias, the latter often by intravenous bolus administration or short infusion. Early studies of this type of administration showed a delay between the concentration of verapamil in venous blood and its effects on A-V conduction in some circumstances (McAllister and Kirsten, 1982) but not others (McAllister et al., 1977). Attempts to account for this delay (hysteresis) by measuring the myocardial concentrations of verapamil have had mixed success. In dogs, verapamil concentrations in direct myocardial biopsies were linearly related to the time course of its myocardial concentrations. Thus, the observed hysteresis for these effects compared with arterial blood was largely caused by the time required for the myocardial equilibration. The model predicted that the myocardial concentrations of verapamil were relatively insensitive to the duration of injection of a given bolus dose, but that rapid injection caused transient, high arterial concentrations. It also predicted that the bolus dose of verapamil should be modified over a 2-fold range to account for physiologically plausible variations in base-line cardiac output and myocardial blood flow.

Several developments have contributed to the understanding of these earlier studies, and suggest the following hypotheses. First, like many other drugs (Huang et al., 1993; Upton et al., 1996), it can be hypothesized that the myocardial effects of verapamil are directly related to its concentration in the myocardium. Second, it is now clear that the enantiomers of verapamil can differ in their kinetics and dynamics in the myocardium. First, like many other drugs (Huang et al., 1993; Upton et al., 1996), it can be hypothesized that the myocardial effects of verapamil are directly related to its concentration in the myocardium. Second, it is now clear that the enantiomers of verapamil can differ in their kinetics and dynamics in the myocardium.
sized that this alone could account for the hysteresis observed in some studies in which only racemic verapamil was assayed. Third, it could be hypothesized that recent insights into the kinetics of bolus administration (Upton, 1996), when applied to verapamil, would provide greater insight into the relationship between verapamil dose and duration of injection and the amount of verapamil entering the heart. Important processes in bolus kinetics are the mixing of drug with venous blood and cardiac output (Upton and Huang, 1993), the kinetics of the first-pass passage of the drug through the lungs (Roerig et al., 1989) and the kinetics and dynamics of the drug in its target organ, in this case the heart (Upton, 1996; Huang et al., 1993). Indeed, descriptions of initial bolus kinetics that do not include these processes but consider bolus administration as the addition of a drug to a central compartment often perform poorly, with very unreliable estimates of the central volume (Keefe and Kates, 1982).

In this paper, we examine these hypotheses by measuring the enantiomer-specific lung, heart and systemic kinetics of verapamil, and its myocardial dynamics, in a conscious chronically instrumented sheep preparation. We identify the determinants of the myocardial concentrations and effects of verapamil and propose a physiologically based pharmacokinetic-pharmacodynamic model of the process [analogous to our previous work with propofol (Upton and Ludbrook, 1997; Ludbrook and Upton, 1997)]. Simulations with the model are used to provide important insights into the dose requirements of verapamil.

Methods

The study protocol was approved by the institutional Animal Ethics Review Committee. Seven adult merino ewes weighing approximately 50 kg were prepared with chronic intravascular catheters and Doppler flow probes to allow drug administration, cardiovascular function monitoring and the measurement of blood flows.

Animal Preparation

The method for preparing the sheep has been described in detail elsewhere (Huang et al., 1992). Sheep were prepared under anesthesia with ultrasonic Doppler flow probes on the left main coronary artery (for measurement of an index of left myocardial blood flow) and the trunk of the pulmonary artery (for measurement of cardiac output). The calibration of these flow measurements also has been described previously (Huang et al., 1992). Intravascular catheters were placed via the right carotid artery or jugular vein in the ascending aorta (for arterial blood sampling and placement of a left ventricular manometer catheter), inferior vena cava (for drug administration), the coronary sinus (after ligation of the hemiazygous vein for sampling myocardial effluent blood) and in the pulmonary artery (for blood sampling). All the surgical procedures were performed by sterile technique. After they recovered from anesthesia, the sheep were placed in mobile metabolic crates and their catheters were flushed continuously with heparinized (5 IU/ml) 0.9% saline at a rate of 3 ml/hr, with a gas-powered system (Runciman et al., 1984). All animals had free access to food and water.

Hemodynamic Measurements

On an experimental day, the following hemodynamic measurements were made by methods reported previously (Huang et al., 1992). Myocardial blood flow and cardiac output were derived from the output of the Doppler flow probes. An index of myocardial contractility was derived from the LV dP/dt max, recorded by use of an acutely placed micromanometer catheter in the left ventricle. Mean arterial blood pressure was measured with a pressure transducer on one of the arterial catheters. These hemodynamic parameters were recorded with an analog-to-digital card (Metabyte DAS 16-G2) and a personal computer (486 IBM compatible). A quadrupolar electrocardiogram was recorded from electrodes placed on each leg of the sheep and on a high-frequency response chart recorder (Devices 4 channel). This electrocardiogram was later analyzed to determine the heart rate and PR interval to give an index of the rate of A-V conduction.

Study Design and Pharmacokinetic Measurements

Studies were conducted in seven sheep prepared as described above. After the placement and the calibration of the hemodynamic measurement devices, sheep were allowed to "settle down" for approximately 30 min before base-line measurements of the hemodynamic parameters were recorded. The sheep were administered 10-mg doses of verapamil hydrochloride (Isoptin 5 mg/2 ml, Knoll AG, Germany) diluted with 0.9% saline (total volume, 20 ml) as a 2-min i.v. infusion into the right atrium. During the experiments, the sheep were partially supported in a comfortable sling inside their metabolic crates to prevent them from lying down during the study, which would influence the hemodynamic measurements. After the start of the verapamil infusion, the hemodynamic parameters were recorded continuously for the next 30 min, and pulmonary arterial and coronary sinus blood samples (1 ml) were taken at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5 and 30 min by methods reported previously by our laboratory (Huang et al., 1991).

Verapamil Assay

Verapamil was assayed by a high-performance liquid chromatography method based on enantiomer separation with a Chiral-AGP column (100 × 4.0 mm, ChromTech AB, Hagersten, Sweden), as described in the application notes for this column (ChromTech Application note no. 11 (1993), ChromTech AB, Hagersten, Sweden). The whole-blood samples were collected into 10-ml glass tubes that contained 0.25 μg of (+)-mepivacaine as an internal standard and 21 μl of heparin (1000 IU/ml) as an anticoagulant. They were processed by extraction into diethyl ether under basic conditions, followed by acidic extraction into 200 μl of 0.005 N phosphoric acid. This acidic phase (50 μl) was injected into the high-performance liquid chromatography which used a buffer containing 90% 0.01 N Na2HPO4 (pH 7.15) and 10% acetonitrile. Detection was by ultraviolet absorbance at 214 nm. A runtime of approximately 15 min separated the enantiomers of verapamil, and the limit of sensitivity was approximately 0.02 μg/ml.

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To determine whole-blood verapamil concentrations, five-point standard curves (range, 0.05–0.5 μg/ml) were prepared in drug-free whole blood taken from the animal immediately before the study. The mean R² values of the standard curves were 0.9992 (S.D. 0.0008) and 0.9996 (S.D. 0.0002) for the (+)- and (−)-enantiomers, respectively.

Kinetic and Dynamic Analysis

In general terms, lung kinetic values were deduced from the pulmonary artery-arterial verapamil gradient and cardiac output, whereas heart kinetic values were deduced from the arterial-coronary sinus verapamil gradient and myocardial blood flow. Systemic kinetic values were deduced from the time course of the arterial verapamil concentrations. The dynamic effects of verapamil on myocardial contractility (LV dP/dt max), myocardial blood flow and A-V conduction (PR interval) were compared with the concentrations of verapamil in arterial or coronary sinus blood. The complex relationships between these kinetic and dynamic processes were synthesized by physiological modeling as described below.

Modeling methods. The general modeling method was the hybrid modeling of kinetics and/or dynamics in separate regions (e.g., lung, heart) with empirical forcing functions to represent inputs into the region and curve-fitting of the output to determine model parameters (Upton, 1996). Curve-fitting was by a least squares method...
based on the maximization of MSC, which is essentially the Akaike Information Criterion scaled to compensate for data sets of different magnitudes (Wagner, 1993). In this context, values of about 5 are consistent with an extremely good fit of the data, whereas values of about 1 are consistent with a poor fit. No weighting was considered necessary because there was no evidence that the data were heteroscedastic. Models were constructed as a series of differential equations with the Scientist for Windows software package (Version 2, Micromath Scientific Software, Salt Lake City, Utah). Models were fitted to mean data for the seven sheep.

Lung models of each enantiomer. The measured pulmonary artery verapamil concentrations \(C_{pa}\) and cardiac output \(Q_{co}\) data were fitted to forcing functions (exponential and polynomial functions, respectively) and these were used as the input functions for the models. The measured arterial concentrations \(C_{art}\) were used to estimate the parameters of the following models by curve-fitting for each enantiomer.

1. A single flow-limited compartment with the mass balance equation modified for effluent rather than tissue drug concentrations, where \(V_{lung}\) is the apparent volume of the compartment representing the lung.

\[
V_{lung} \frac{dC_{art}}{dt} = Q_{co} \cdot (C_{pa} - C_{art})
\]

(1)

2. A single flow-limited compartment with first-order lung extraction (\(ER_{lung}\)):

\[
V_{lung} \frac{dC_{art temp}}{dt} = Q_{co} \cdot (C_{pa} - C_{art temp})
\]

\[
C_{art} = C_{art temp} \cdot (1 - ER_{lung})
\]

(2)

3. A membrane-limited model where "PS" is used to represent membrane permeability in keeping with standard principles of capillary exchange (Intaglietta and Johnson, 1978) and \(C_d\) is the verapamil concentration in the deep compartment of the lung with a volume given by \(V_{deep}\).

\[
V_{lung} \frac{dC_{art}}{dt} = Q_{co} \cdot (C_{pa} - C_{art}) + PS \cdot (C_{d} - C_{art})
\]

\[
V_{deep} \frac{dC_{d}}{dt} = PS \cdot (C_{art} - C_{d})
\]

(3)

Heart models of each enantiomer. The process used was similar to that described above for the lung, but the arterial concentrations of verapamil and myocardial blood flow data were fitted to forcing functions and used as the inputs, and the coronary sinus concentrations were used as the output for parameter estimation. The structural models examined for each enantiomer were those described above for the lung, but to define the model in terms of parameters more relevant to the heart than the lungs, the flow-limited compartment with extraction was expressed alternatively as small first-order loss from the compartment:

\[
V_{h} \frac{dC_{CS}}{dt} = Q_{h} \cdot (C_{art} - C_{CS}) - k_{out} \cdot C_{CS}
\]

(4)

\(C_{art}\) is the arterial verapamil concentration, \(C_{CS}\) is the coronary sinus verapamil concentration, \(Q_{h}\) is myocardial blood flow, \(V_{h}\) is the apparent volume of the heart and \(k_{out}\) is the rate constant of drug loss. The model was expressed in this form for comparison with the reported loss of some drugs from the surface of the heart into surrounding fluids (Huang and Upton, 1993).

Systemic kinetics model. The systemic kinetics of each enantiomer of verapamil was modeled as two-tissue pools, representing well perfused tissues (other than the heart and lung) and poorly perfused tissues, respectively. These were connected in a recirculatory manner with the lung and heart models as shown in figure 1. A vascular mixing compartment was included with parameters based on previous work (Upton, 1996), which improves the description of bolus kinetics. The fraction of cardiac output (less myocardial blood flow) to each tissue pool was set at 80% and 20%, respectively. It was necessary to separate the enantiomers because they have different potency ratios, as shown in table 4.

Fig. 1. The overall structure of the model. The model is composed of six compartments representing a venous mixing, lung, deep lung, heart and two tissue pools connected in a recirculatory manner through which the cardiac output \(Q_{co}\) flows. The kinetics of the (+)- and (-)-enantiomers of verapamil were treated separately in each compartment. The effects of verapamil on myocardial blood flow \(Q_{h}\), myocardial contractility (LV dP/dt\(_{max}\)) and A-V conduction (PR interval) were treated as the sum of the effect of both enantiomers using the potency ratios shown in table 4.
necessary to set a value for this fraction term to prevent the values of the tissue pool volumes from being underdetermined in the fitting process. A first-order clearance process from the well perfused compartment nominally represented hepatic clearance. To determine the values of the parameters of these tissue pools in the combined model (which included previously estimated model parameters for the lungs and heart), they were curve-fitted simultaneously to the arterial and pulmonary concentration data for each enantiomer.

Pharmacokinetic-pharmacodynamic analysis for each enantiomer. Semiparametric first-order effect compartment analysis was used to determine the first-order rate constant ($k_{oa}$) relating the time course of the arterial or coronary sinus blood concentrations of each enantiomer and the time course of three drug effects (LV dP/dt$_{max}$, PR interval and myocardial blood flow) by methods reported previously (Upton et al., 1996). The arterial or coronary sinus concentrations ($C_{oa}$) were fitted to forking functions, and the effect compartment concentration ($C_{eff}$) was given by the following equation:

$$\frac{dC_{eff}}{dt} = k_{oa}(C_{bl}-C_{eff})$$  (5)

Linear, log-linear, $E_{max}$ and sigmoid $E_{max}$ dynamic models relating $C_{eff}$ to the observed effect ($E$) were compared in each case based on the following equations:

$$E = \text{base line} \pm \text{slope} \cdot C_{eff}$$  (6)

$$E = \text{base line} \pm \frac{E_{max} \cdot C_{eff}}{EC_{50} + C_{eff}}$$  (7)

$$E = \text{base line} \pm \frac{E_{max} \cdot C_{eff}}{EC_{50p} + C_{eff}}$$  (8)

where $E_{max}$ is the maximum drug effect, and $EC_{50}$ is the concentration at which half the maximum effect occurs, base line is the base-line level of effect, and the + or - term is used if the drug increases or decreases the effect relative to the base line, respectively. The fitted effect compartment rate constants were converted to half-lives for further analysis. If a drug effect preceded a drug concentration, the hypothetical delay was estimated by fitting the concentrations to the effects by the inverse of the dynamic function.

For example, the inverse of equation 7 is as follows:

$$C_{eff} = \frac{(\text{base line} - E)EC_{50}}{E_{max} - (\text{base line} - E)}$$  (9)

Combined kinetic-dynamic model for both enantiomers. Although data on the relationships between the concentrations of each enantiomer of verapamil and their combined effect on the heart, from the previous sections are useful for choosing the concentration site best related to a drug effect, conclusions drawn from these data can be misleading if potency resides predominantly in one enantiomer, as for verapamil (Satoh et al., 1980). The combined kinetic-dynamic model was therefore structured as follows: After the administration of racemic verapamil, the kinetics of each enantiomer was handled separately in each organ of the model as described above. The measured drug effects were assumed to be the sum of the effect caused by the concentration of each enantiomer at the effect site of relevance, with the difference in potency accounted for by differences in the $EC_{50}$ of each enantiomer. The following equations are an example, and have been used to relate the LV dP/dt$_{max}$ changes (dpdt) to the coronary sinus concentrations of each enantiomer [$C_{com}$ for the (-)-enantiomer, $C_{exp}$ for the (+)-enantiomer]. $E_{max}$ is the maximum drug effect, $EC_{50}$ is the concentration at which half the maximum effect is achieved for the (-)- and (+)-enantiomers, respectively, and $P$ is the potency ratio (-)/(+).$EC_{50p} = \frac{EC_{50m}}{P}$

$$dpdt = \text{base line} - \left( \frac{E_{max} \cdot C_{com}}{(EC_{50m} + C_{com})} \right) - \left( \frac{E_{max} \cdot C_{exp}}{(EC_{50p} + C_{exp})} \right)$$  (10)

This equation requires the estimation of only three parameters ($E_{max}$, $EC_{50m}$ and base line) from the concentration and effect data.
TABLE 1
Lung kinetic models

The parameters of the flow-limited (flow), flow-limited with extraction (flow + ER) and membrane-limited (membrane) lung kinetic models for each enantiomer. Goodness of fit is given by the $R^2$ value and the MSC (see the text). A higher MSC indicates a better fit. The data are shown as the mean and upper and lower 95% confidence limits. An asterisk indicates that the value for the (+) enantiomer was statistically different from that for the (-) enantiomer by comparison of the mean with the 95% confidence limits.

<table>
<thead>
<tr>
<th>Enantiomer</th>
<th>$V_{\text{lung}}$ (l)</th>
<th>ER$_{\text{lung}}$</th>
<th>PS (l/min)</th>
<th>$V_{\text{deep}}$ (l)</th>
<th>$R^2$</th>
<th>MSC</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow</td>
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<tr>
<td>Flow + ER</td>
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<td>4.93</td>
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<tr>
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<td>2.14</td>
<td>5.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)-Enantiomer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow</td>
<td>5.27</td>
<td>0.951</td>
<td>2.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow + ER</td>
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<td>0.996</td>
<td>5.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>4.78*</td>
<td>2.29</td>
<td>5.29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results

Blood concentration differences between the enantiomers of verapamil. The time courses of the concentrations of each enantiomer of verapamil in pulmonary artery, arterial and coronary sinus blood were superficially similar and are shown in figure 2. However, 95% confidence limits showed that concentration of the (+) over the (-) concentration was greater than that of the (-) for each site at some stage during the 30-min study period (fig. 3), which suggests that an enantiomer-specific analysis was necessary. The times of the peak arterial, pulmonary artery and coronary sinus concentrations were 2, 2 and 4.5 min, respectively, and did not differ between enantiomers.

Lung kinetics of verapamil. The parameters and "goodness of fit" of the various models are shown in table 1. The flow-limited model was a poor fit of the data, principally because less verapamil left the lungs than entered during the experimental period. This could be addressed by adding a first-order extraction term of 22 to 26%, but a better fit was achieved by sequestering this "missing drug" in a relatively large "deep distribution" compartment in the membrane-limited model. This provided an excellent fit of the data (fig. 4) and good estimates of parameters. The ratio of permeability (PS, approximately 2 l/min) over flow (cardiac output, approximately 5.6 l/min) of approximately 0.3 places this model in the category that is both flow and membrane limited (Piiper and Scheid, 1981). The volume of this deep compartment differed between enantiomers.

Myocardial kinetics of verapamil. All three models were good descriptions of the data, with general agreement that the volume of the heart was about 1.1 l (table 2). A subtle improvement was gained by including the first-order loss, which was relatively small. However, this loss was not consistent with distribution into a deep compartment of the membrane-limited model, which gave a slightly worse fit and under-determined parameters (table 2). The first-order loss model provided a good fit of the data (fig. 5) and was used for the subsequent systemic modeling, this loss may be caused...
The effect delay half-lives for various concentrations and to 6 min, with a peak increase of 131% of base line at 3.5 min. similar period. Heart rate was increased significantly from 1 increased transiently to a minimum of 90% of base line for a period from 0.5 to 2 min, whereas blood pressure was de-maximum of 122%, but was only statistically increased for a pronounced. Cardiac output was increased transiently to a base line, respectively. Its hemodynamic effects were less blood flow and PR interval increased to 213% and 141% of creased to 45% of base line at 3 min, whereas myocardial limits. There were no statistically significant differences between the means for each enantiomer by comparison of the means and 95% confidence limits.

Fig. 5. The line of best fit (solid line) of the single flow compartment with small first-order loss model of myocardial kinetics for the (-)-enantiomer to the observed coronary sinus (-)-verapamil concentrations (mean, open circles; 95% confidence limits, dotted lines).

by diffusion of verapamil from the surface of the heart into pericardial fluid, which has been reported for other drugs (Huang and Upton, 1993). The differences in myocardial kinetics between the enantiomers were insignificant (table 2).

Systemic kinetics of verapamil. For the (+)-enantiomer, the volumes (mean ± S.D.) of tissue pool 1, tissue pool 2 and the total clearance where 10.09 ± 2.28 l, 51 ± 66 l and 2.82 ± 0.87 l/min, respectively. For the (-)-enantiomer, these values were 9.87 ± 2.49 l, 38 ± 15 l and 2.70 ± 0.77 l/min. With the exception of the volume of tissue pool 2 for the (+)-enantiomer, these were precise estimates, and the MSC was relatively good (2.92 for the (+) and 3.01 for the (-)) for each enantiomer.

Pharmacodynamic effects of verapamil. Verapamil had profound effects on the heart (fig. 6). LV dP/dt max decreased to 45% of base line at 3 min, whereas myocardial blood flow and PR interval increased to 213% and 141% of base line, respectively. Its hemodynamic effects were less pronounced. Cardiac output was increased transiently to a maximum of 122%, but was only statistically increased for a period from 0.5 to 2 min, whereas blood pressure was decreased transiently to a minimum of 90% of base line for a similar period. Heart rate was increased significantly from 1 to 6 min, with a peak increase of 131% of base line at 3.5 min.

Pharmacokinetic-pharmacodynamic relationships. The effect delay half-lives for various concentrations and effects are shown in table 3. The changes in Qh were well-related to the time course of the arterial concentrations of each enantiomer, whereas the changes in PR interval were well related to the time courses of their coronary sinus concentrations. The LV dP/dt max data were problematical in that the analysis showed they were slightly delayed relative to the arterial concentrations, but preceded the coronary sinus concentrations by a smaller amount (table 3), although the coronary sinus concentrations were substantially delayed relative to the arterial. This may have been because the time course of LV dP/dt max did not have a well defined minimum value, and was at its minimum for a period of 2 to 3 min. Hysteresis analysis was conducted by quantitating the areas under the curve of the ascending and descending limbs of concentration-effect plots (Huang et al., 1993). This showed that hysteresis was considerably smaller for the coronary sinus concentrations than for the arterial, so in subsequent analysis the changes in LV dP/dt max were assumed to be a function of the coronary sinus (and therefore myocardial concentrations).

Combined kinetic-dynamic model. An E max model was the most appropriate dynamic model for the combined effects of each enantiomer on Qh, LV dP/dt max and PR interval, giving the highest value of the MSC with precise estimation of parameters (table 4). The fits of the best models to the effect data are shown in figure 6.

Potency ratios of 5:1, 8:1, 10:1 and 12:1 for the effect of (-)(+)-verapamil on LV dP/dt max in the combined kinetic-dynamic model gave MSC values of 5.21, 5.27, 5.28 and 5.17, respectively, with all ratios showing good fits of the observed data. Thus, the predictions of the model are relatively insensitive to the arterial. This may have been because the time course of LV dP/dt max did not have a well defined minimum value, and was at its minimum for a period of 2 to 3 min. Hysteresis analysis was conducted by quantitating the areas under the curve of the ascending and descending limbs of concentration-effect plots (Huang et al., 1993). This showed that hysteresis was considerably smaller for the coronary sinus concentrations than for the arterial, so in subsequent analysis the changes in LV dP/dt max were assumed to be a function of the coronary sinus (and therefore myocardial concentrations).

Implications of the model. The effect of altering the duration of administration of the same dose is shown in figure 7. The peak coronary sinus concentrations were delayed behind the end of injection by nearly 3 min for a 10-sec injection, but only 1 min for a 480-sec injection. For durations between 10- and 240-sec injections, the peak concentrations were within 95% of each other, whereas the value was 90% for the 480 sec injection. More rapid injections were associated with high transient arterial concentrations (fig. 7).

The results of the simulation of the effect of flow changes on dose requirements is shown in figure 8. Note that changes

<table>
<thead>
<tr>
<th>(+)-Enantiomer</th>
<th>(-)-Enantiomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow</td>
<td>1.11</td>
</tr>
<tr>
<td>Flow + loss</td>
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Fig. 5. The line of best fit (solid line) of the single flow compartment with small first-order loss model of myocardial kinetics for the (-)-enantiomer to the observed coronary sinus (-)-verapamil concentrations (mean, open circles; 95% confidence limits, dotted lines).
concentration (LV dP/dt max) and PR interval as given in Table 3. The data are shown as the mean and upper and lower 95% confidence limits. A negative effect half-life indicates that the effect preceded the concentration indicated. An asterisk indicates that the value for the (+)-enantiomer was statistically different from that for the (-)-enantiomer by comparison of the mean with the 95% confidence limits.

**Discussion**

Keefe and Kates (1982) have previously modeled the myocardial disposition of verapamil in dogs, and Powell et al. (1990) have examined the myocardial uptake of verapamil in humans. However, the present data apparently are the first in which myocardial uptake has been examined in an enantiomer-selective manner.

**A comparison of the kinetics of (-)- and (+)-verapamil.** By nature, 10 mg of racemic verapamil contains 5 mg of each enantiomer. Therefore, in the absence of enantiomer-specific kinetics, the time courses of the concentrations of each enantiomer will be the same. Figure 1 shows that in broad terms this was the case. Modeling, however, revealed differences in the extent of deep distribution of the enantiomers into the lungs (table 1). This may have been sufficient to account for the observed differences in the time courses of each enantiomer (fig. 3).

Although the gross similarity in the kinetics of the enantiomers in this study simplifies the interpretation of the present data, other studies clearly show that it is impossible to generalize about the importance of stereoisomerism for verapamil, because there are substantial species differences. Eichelbaum et al. (1984) reported that there were significant differences in the protein binding and the total systemic plasma clearance of (-)- and (+)-verapamil in humans. The difference in clearance presumably was caused by hepatic clearance, because oral administration resulted in significantly higher concentrations of (+)-verapamil. Recently, Laethem et al. (1995) examined the kinetics of the enantiomers of verapamil in rabbits and dogs. Like the sheep data presented here, there were no great differences in kinetics in the rabbit, whereas enantiomeric differences in the dog were caused by differences in protein binding and metabolism which may resemble more closely the situation in humans.

There appears to be more consistent data regarding the differences in the dynamic effects of (-)- and (+)-verapamil, with most studies showing that each enantiomer has similar qualitative effects, but with the (-)-enantiomer showing greater potency.

**The kinetic role of the lung.** The lung plays an important role in bolus kinetics (Chiu, 1979; Jones and Nicholas, 1981; Roerig et al., 1989), whereby it can act as a "capacitor" for a drug between the injection site and its target organ. The greater the storage capacity of the lung, the greater the ability of the lung to damp the rapidly changing pulmonary artery blood concentrations caused by bolus injection (Upton and Huang, 1993). This appears to be the case for verapamil, for which a membrane-limited model with significant distribution volumes was found. This is compatible with the significant uptake (50%) of verapamil reported for the human lung (Roerig et al., 1989).
Heart kinetics. The apparent volume of the heart for both (-)- and (+)-verapamil was approximately 1.1 l. Given that the mass of the heart tissue drained by the coronary sinus in sheep has been measured as 216 ± 37 g (Huang, 1991), this apparent volume equates to a heart/blood partition coefficient of approximately 5.1, which is similar to the value of 6.2 reported in dogs (Keefe and Kates, 1982) and 7.05 in humans (Padrini et al., 1985).

The apparent volume of verapamil in the heart is relatively large compared with the base-line myocardial blood flow (0.094 l/min) and equates to a mean transit time of approximately 12 min, and an arterial blood/myocardial equilibrium half-life (Runciman and Upton, 1994) of approximately 8 min. The consequences of this delay between the arterial concentrations of verapamil and its concentrations in the heart would be most significant after bolus administration of less than 1-min duration, and should be accounted for when titrating verapamil to an observable myocardial effect (e.g., ECG changes) as the peak myocardial concentration (and therefore effect) will occur some 3 min after the injection for slower injections and short infusions (e.g., 8-min duration), this delay in peak effect was reduced to approximately 1 min after the end of the injection.

Kinetic-dynamic relationships. The importance of the myocardial concentration of verapamil in determining the magnitude of its effects on myocardial contractility and conduction has been shown in vitro (Chiba et al., 1978; Raschack, 1976) and in vivo (Gloor and Urthaler, 1983), and it has been shown that it is the concentration of the unionized form that is important in determining effects (Cohen et al., 1987). These data support the results of the present study and the use of the myocardial concentrations as the determinant of myocardial effects in the model. The fact that the changes in myocardial blood flow were better related to the time course of the arterial concentrations is intriguing. This suggests that flow changes are dictated by the concentration of verapamil in the arteries and arterioles of the heart, which rapidly equilibrate with arterial

<table>
<thead>
<tr>
<th>Effect</th>
<th>Potency Ratio (-)/(+)</th>
<th>Base line</th>
<th>E_{max}</th>
<th>EC_{50} (-)</th>
<th>MSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q_{h}</td>
<td>2</td>
<td>0.093</td>
<td>0.109</td>
<td>0.14</td>
<td>4.19</td>
</tr>
<tr>
<td>LV dP/dt_{max}</td>
<td>4</td>
<td>2659</td>
<td>1445</td>
<td>0.64</td>
<td>5.05</td>
</tr>
<tr>
<td>PR interval</td>
<td>10</td>
<td>9.59</td>
<td>5.31</td>
<td>0.045</td>
<td>5.58</td>
</tr>
</tbody>
</table>

Table 4
Combined effect model parameters
The parameters of the best dynamic models for changes in myocardial blood flow (Q_{h}), the maximum rate of rise of left ventricular pressure (LV dP/dt_{max}) and A-V conduction (PR interval) relative to arterial (Q_{h}) and coronary sinus blood (LV dP/dt_{max} and PR interval) for both the (+)- and (-)-enantiomers based on the potency ratios shown. An example of the equations of the dynamic models is shown in the text. The data are shown as the mean and upper and lower 95% confidence limits.
blood, rather than in the tissue of the heart that equilibrate more slowly.

The half-life of the delay or hysteresis between verapamil blood concentrations and effects have been reported to range from approximately 2 min (Schwartz et al., 1989) to 49 min (Colburn et al., 1986). However, when arm venous blood samples are used (Schwartz et al., 1989), the delay in arm/blood equilibrium fortuitously may be similar to that of myocardial/arterial blood equilibrium, giving rise to negligible delay. Unfortunately, this approach may fail when altered physiological conditions cause changes in myocardial but not arm blood flow, and vice versa. The present data suggest that the effect delay for verapamil (for contractility and A-V conduction) is consistent with the delay caused by myocardial/arterial blood equilibrium.

Dose implications. The predictions of the model provide insight into the determinants of the myocardial uptake of verapamil, but still must be confirmed experimentally. They suggest, however, that the myocardial concentrations of verapamil are relatively insensitive to the duration of injection of a given dose because of the “damping” of the injected peak in the relatively high distribution volumes of the lungs and heart. The common clinical practice of infusion of a given dose of verapamil for 5 min is confirmed by these data. For this duration of injection, the peak myocardial concentrations could be expected to occur between 1 and 2 min after the end of the infusion. Thus, when titrating verapamil against a clinical indicator, this is predicted to be the minimum interval between doses required so that another dose is not injected before the maximum effect of the previous dose has been manifested.

Injections of the same dose more rapidly than during 5 min do little to hasten the entry of verapamil into the heart but are associated with high transient arterial concentrations, which may have adverse effects of their own (Ludbrook and Upton, 1997) and allow less time for homeostatic control mechanisms to compensate for changes in the circulatory state. Conversely, infusion of the same dose during periods longer than 5 min will cause reductions in peak myocardial concentrations because of greater elimination and redistribution of verapamil before the peak myocardial concentration.

Of more importance with respect to dose requirements of verapamil is the cardiac output and myocardial blood flow of an individual. The model predicts that variability in the myocardial effects of verapamil between and within individuals could be improved by adjusting the dose for the anticipated cardiac output and myocardial blood flow of the individual. This is consistent with previous reports of cardiac output dependent kinetics for other drugs (Christensen et al., 1982; Henthorn et al., 1992; Krejcie et al., 1994; Watt et al., 1996), but awaits experimental confirmation for verapamil in humans and other species.

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References


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