Assessment of hepatic blood flow using continuous infusion of high clearance drugs

R. C. Schoemaker, J. Burggraaf & A. F. Cohen

Centre for Human Drug Research, Leiden, The Netherlands

Aims To provide methods for the translation of the concentration–time profile of highly cleared marker compounds into the underlying clearance and hepatic blood flow profile.

Methods Continuous infusion of indocyanine green or sorbitol was used to assess the effect of the hepatic blood flow modifiers exercise, somatostatin and octreotide. Three distinct methods are described for the translation of concentration into flow:

1. assuming successive phases of constant clearance
2. point to point estimation of clearance using estimates of concentration change
3. using a parametric description of the flow profile in combination with the differential equations describing the change in marker concentrations.

Results The marker compound concentration profiles are adequately described using the different methods. Exercise results in a decrease in hepatic blood flow of about 80%. Somatostatin and octreotide elicit an indistinguishable hepatic blood flow decrease from 1.49 to 1.07 l min⁻¹. Return to baseline takes much longer for octreotide (half-life 126 ± 104 min) than for somatostatin (half-life 4.29 ± 3.55 min).

Conclusions Translation of concentration profiles into clearance profiles is possible making continuous assessment of hepatic blood flow feasible.

Keywords: hepatic blood flow, continuous infusion, indocyanine green, sorbitol, exercise, somatostatin, octreotide

Introduction

Drugs with high hepatic clearance depend on the rate of blood flow through the liver for their elimination from the system. As many of these drugs are on the market and many more are under development, knowledge of the rate of hepatic blood flow may be essential in the assessment or prediction of drug action. Additionally, assessment of changes in hepatic blood flow may be the focus of research into the (patho)physiology of the liver.

A number of techniques have been successfully employed to assess hepatic blood flow. This paper will focus on a kinetic approach that uses the concentration–time profile of drugs with predominant hepatic clearance to provide information on hepatic blood flow [1].

Traditional kinetic analysis techniques using intravenous bolus administration must assume that the physiologic conditions and the associated pharmacokinetic model remain stationary. Therefore, bolus injections will only provide average flow estimates over the period that concentrations are measured, and timing of injections is critical when investigating short lasting changes in hepatic blood flow. On the other hand, continuous assessment of the effects of changing hepatic blood flow on plasma concentration is possible, by frequent monitoring of plasma concentrations during a zero-order infusion of a marker compound [2]. The resulting concentration profile must however still be translated into a flow profile; an abrupt change in flow for example will result in a more gradual change in concen-

tration, governed by the half-life of the marker compound. This paper describes three closely related ways of extracting information on hepatic blood flow from concentration profiles. This is illustrated using two examples with the marker compounds indocyanine green and sorbitol, displaying different kinetic behaviour and requiring different solutions. The emphasis of this paper is on methodology and the presented examples serve as illustration only; clinical results may be reported elsewhere.

Methods

Kinetics during infusion

The plasma concentration–time profile (C_t) for drugs that can be described by a one-compartment open model during a constant rate infusion (with rate R_{inf}) is given by the usual formula:

$$C_t = \frac{R_{inf}}{CL} (1 - e^{-t \cdot CL/V}) \quad (1a)$$

where t is the time after the start of the infusion, CL is plasma clearance and V the volume of distribution. After the infusion stops (after T min), the decline in concentration is described by:

$$C_t = \frac{R_{inf}}{CL} (1 - e^{-T \cdot CL/V}) e^{-(t-T) \cdot CL/V} \quad (1b)$$

Clearance and volume are assumed constant. The relationship between hepatic plasma clearance and hepatic blood flow

(Q) is given by:

$$CL = (1 - Ht) Q \cdot E \quad (2)$$

where E is the extraction ratio and Ht is the haematocrit needed to translate blood volume to plasma volume. There are a number of models describing the relationship between hepatic blood flow and extraction ratio. The two oldest, most simple and best known are the parallel-tube or undistributed sinusoidal model and the well-stirred or venous equilibration model [3, 4]. More advanced models (like the dispersion, series-compartment and distributed sinusoidal models) provide predictions intermediate to the two basic models. Therefore, the predictions of the two basic models represent the two opposite extremes of the predictions of all the available models [4]. The two basic models differ significantly in their predictions for drug behaviour after oral administration and provide very different estimates for intrinsic clearance (related to enzymatic capacity in the liver) at the same extraction ratio. Nevertheless, the shape of the predicted nonlinear relationship between hepatic blood flow and extraction ratio is similar (see Figure 1) and in practice it is difficult to determine which of the models provides the most adequate description [3]. The data presented in this paper do not in any way allow discrimination between the different extraction models; this would require direct assessment of extraction ratios and actual hepatic flow. For the well-stirred model the form of this relationship is:

$$E = \frac{f_B'' CL_{int}}{f_B'' CL_{int} + Q} \quad (3a)$$

and for the parallel tube model it is:

$$E = 1 - e^{f_B'' CL_{int}/Q} \quad (3b)$$

where f_B'' is the fraction of unbound drug, and CL_{int} is the intrinsic clearance of the liver, related to the metabolic capacity of the liver enzymes for the cleared drug. If the haematocrit and extraction ratio at a particular flow are known, clearance can be translated into flow. The two

marker compounds that figure in this paper have different extraction ratios. The first is indocyanine green (ICG), an inert green dye used for decades in the context of hepatic blood flow estimation, with an extraction ratio of about 0.7 for healthy volunteers with a liver blood flow of around 1.5 l min^{-1} [1, 5]. The second is sorbitol that has a much higher extraction ratio of around 0.96 [5]. The difference in extraction ratios results in a more linear relationship between hepatic blood flow and plasma clearance for sorbitol than for ICG (Figure 1). This figure also illustrates that for flows below normal values, the relationships are virtually indistinguishable between the models.

Hepatic blood flow is seldom constant. Changes can be induced by posture, exercise, food intake, vasoactive compounds and many other circumstances [6]. This means that for a drug with a high extraction ratio, equations (1a) and (1b) are rarely applicable. The kinetics of a drug with one-compartment properties can however also be characterized using a differential equation that describes changes in concentration (dC_t/dt) as a function of infusion rate, drug concentration itself and the relevant kinetic parameters:

$$\frac{dC_t}{dt} = \frac{R_{inf}}{V} - \frac{CL_t}{V} C_t \quad (4)$$

For drugs with two-compartment pharmacokinetic properties, a system of two differential equations is required. Clearance is no longer required to be constant in these equations but may change over time (CL_t). The solution to this differential equation is given by the predicted concentration-time profile. For constant clearance, this solution is provided by equations 1a and b. For clearance that changes over time, an explicit solution cannot be obtained in general and parameters have to be estimated using iterative computational techniques. However, for a limited number of clearance-time patterns an explicit solution can be obtained, as is demonstrated in the first example.

Example 1: Hepatic blood flow during exercise

The first example is obtained from a study in which the effect of physical exercise on hepatic blood flow was investigated during a 200 min infusion of indocyanine green (ICG). A constant rate infusion (0.75 mg min^{-1}) of ICG was administered to eight healthy volunteers. Seventy minutes after the start of the infusion, after supposedly obtaining ICG steady state, 20 min of moderate physical exercise was applied. A calibrated bicycle ergometer was used, aimed at reaching a heart rate of $150 \text{ beats min}^{-1}$ after 5 min of exercise and $180 \text{ beats min}^{-1}$ at the end of the 20 min exercise period. Subjects remained seated from at least 30 min prior to the start of ICG infusion until the last blood sample was taken. ICG was measured using h.p.l.c. as previously described [2] in order to avoid problems with accumulation of impurities.

Two different approaches for the translation of ICG concentration-time profiles into ICG clearance-time profiles will be described.

The first approach assumes a succession of three phases with constant clearance during each phase and an instan-

Figure 1 Theoretical relationship between total hepatic blood flow and plasma clearance for ICG (●/○) and sorbitol (■/□); filled markers: well-stirred model; open markers: parallel tube model.

taneous change in clearance from one phase to the next: prior to exercise, during exercise and after exercise. This is one of the rare cases where an explicit solution to differential equation (4) is possible. Equation (1a) may be generalized to the situation where a certain concentration level (C_{t0}) is already present when starting the infusion at t_0 :

$$C_t = \frac{R_{\text{inf}}}{CL} (1 - e^{-(t-t_0)CL/V}) + C_{t0} e^{-(t-t_0)CL/V} \quad (5a)$$

After the infusion has stopped at time T , the decline is given by:

$$C_t = C_T e^{-(t-T)CL/V} \quad (5b)$$

This equation allows the description of the concentration profile associated with the three successive clearance values. Each phase has its own equation, linked to the next phase by the final calculated concentration just before the change from one clearance situation to another. This value is the starting concentration (the C_{t0}) for the next phase. Any nonlinear regression program that allows user-defined models may be used to obtain parameter estimates. The curves from this experiment were analysed using the NLR procedure from SPSS/PC + V4.0.1 (SPSS, Inc., Chicago, IL). Observations were iteratively reweighted by $1/(\text{predicted concentration})^2$ corresponding to the assumption of a constant coefficient of variation for residual error.

The second approach allows an expression for the clearance at each time point by rewriting differential equation (4):

$$CL_t = \frac{R_{\text{inf}} - \frac{dC_t}{dt} V}{C_t} \quad (6)$$

If the volume of distribution (V) is known and constant, then clearance estimates for each time point can be obtained by filling in the corresponding estimate of the rate of concentration change (dC_t/dt) and the measured concentration (C_t) for that time point. Rate of concentration change is given by the first derivative of the concentration-time curve. For this example, the least squares straight line through three adjacent concentration-time points was used to approximate the curve. The slope of this straight line was used as an estimate of dC_t/dt for the middle time point. Volume of distribution was set at the V estimate obtained with the first approach (with the three distinct clearance phases).

Results

Seven subjects completed the treatment; one subject was withdrawn from the study because of signs of phlebitis at the site of ICG infusion. Average ICG concentrations ($\pm$ s.d.) are presented in Figure 2, with the average predicted ICG plasma profile using the 'three clearance phases' model superimposed. Plasma clearance and volume of distribution estimates for this model are presented in Table 1. Hepatic blood flow estimates were calculated for both the well-stirred and the parallel tube models, assuming an extraction ratio of 0.7 at a hepatic blood flow of 1.5 l min^{-1} (meaning $f_B'' \cdot CL_{\text{int}}$ is 3.5 for the well-stirred model and 1.8 for the

Table 1 Parameters for example 1: ICG during exercise assuming three clearances phases.

	Mean	s.d.	Min	Max
<i>Plasma clearance (l min^{-1})</i>				
Prior to exercise	0.632	0.125	0.455	0.843
During exercise	0.145	0.123	0.000	0.299
After exercise	0.632	0.158	0.438	0.890
<i>Volume of distribution (l)</i>				
	5.61	2.01	2.10	8.19
<i>Predicted hepatic blood flow using well-stirred model (l min^{-1}) (assuming $E=0.7$ at $Q=1.5 \text{ l min}^{-1}$)</i>				
Prior to exercise	1.62	0.48	1.01	2.49
During exercise	0.28	0.25	0.00	0.60
After exercise	1.64	0.61	0.96	2.73
Decrease due to exercise	81%	21%	41%	100%
<i>Predicted hepatic blood flow using parallel tube model (l min^{-1}) (assuming $E=0.7$ at $Q=1.5 \text{ l min}^{-1}$)</i>				
Prior to exercise	1.87	1.02	0.91	4.00
During exercise	0.25	0.22	0.00	0.53
After exercise	2.08	1.57	0.86	5.39
Decrease due to exercise	84%	20%	42%	100%

Figure 2 Observed (mean $\pm$ s.d.) plasma ICG profile (—●—●—) and predicted (mean) plasma ICG profile assuming constant clearance for the three phases (—).

parallel tube model) and a haematocrit of 0.42 (Table 1). The resulting flow estimates are different for the two models but if the results are presented as a change in flow during exercise (from prior to exercise) comparable estimates result. These estimates indicate that hepatic blood flow drops by $81\% \pm 21\%$ (well-stirred) or $84\% \pm 20\%$ (parallel tube). Average ($\pm$ s.d.) point to point estimates for ICG plasma clearance calculated using the second approach are presented in Figure 3, with the average 'three clearance phases' estimates superimposed.

Discussion

There are several indications that the assumption of three constant clearances may not be correct. Figure 3 indicates that although the initial drop in clearance is rather abrupt, the return to normal values is more gradual. Also, zero clearance is estimated for two subjects during exercise, and it is unlikely that flow through the liver stops entirely during exercise, although actual extraction could drop to very low levels. Nevertheless, the constant-clearances method is

Figure 3 Calculated ICG plasma clearance. Predictions (mean) assuming constant clearance for the three phases (—) and estimates (mean $\pm$ s.d.) per time point (—●—●—).

appealing if interest lies in estimating average clearance during the three phases. The second approach is less restrictive because it makes no assumptions on the shape of the clearance-time profile and lends itself to the analysis of situations in which no external information is present on the time-profile of the flow-modifier. For instance, the effect of vasoactive drugs or food intake on hepatic blood flow may result in a transient change in flow, but the exact profile or the relationship between drug concentration and effect may be unknown. By translating concentrations into clearances, a point to point assessment of flow may be obtained which can be used to describe the effect profile of the intervention.

Different interpolation strategies may be used to estimate the rate of concentration change. If more than three data points are used in the derivative estimation, the computed clearance profile will be smoother, but consequently abrupt changes will be blunted. Whether or not this is desirable, depends on the anticipated effect profile; an abrupt change may be unphysiological and due to measurement error. Although a simple straight line may seem like a crude approximation to the concentration-time curve, use of smoother functions like parabolas did not result in any improvement. The use of equations other than the straight line will only prove useful if the signal (true change in flow) is much larger than the noise (measurement error in marker concentration), which was not the case in this experiment.

Although ICG is reported to possess multi-compartment properties, no systematic deviations between the predictions using a one-compartment model and measured concentrations after cessation of the infusion were found. We have found no indications that more than single exponential kinetics are required to describe the concentration profile.

The choice of model determines the ultimate hepatic blood flow estimate to a sizeable extent, as does the assumed value for intrinsic clearance. If the ultimate goal is to determine the actual hepatic blood flow, then ICG may not be the best choice for a marker compound. If on the other hand, interest lies mainly in the shape of the flow profile and the percentual changes induced, then the dependency on assumptions is minimised and ICG's more favourable properties (one-compartment kinetics, short half-life) may tip the balance.

External information on the volume of distribution is required to complete calculation of the clearance profile using the second procedure. For the data presented here, the previously obtained V estimate from the first approach was used. In other cases data obtained from literature may provide an approximation. The best procedure however, is probably to precede the continuous infusion by a bolus dose, accompanied by frequent sampling over a limited time span of 10 to 15 min. This allows accurate estimation of the volume of distribution and, as a bonus, steady state concentrations are reached sooner. For ICG infused at 0.75 mg min^{-1} , a bolus dose of 7.5 mg should suffice, provided a sufficiently sensitive assay technique is used.

Example 2: Hepatic blood flow, somatostatin and octreotide

The second example is from a study investigating the effect on hepatic blood flow of the drugs somatostatin and octreotide. The aim was to see if this effect could be equally measured using a continuous infusion of sorbitol as marker-compound and using echo-Doppler flow measurement in an intra-hepatic portal vein. Somatostatin is reported to induce a transient decrease in hepatic blood flow [7, 8]. Octreotide is a somatostatin analogue with a much longer elimination half-life (60 vs 3 min) and has been shown to induce a much longer change in hepatic blood flow [9].

Treatments were administered to six healthy volunteers according to a double-blind randomized crossover design, with treatment order determined using Latin-squares balanced for first-order carry-over effects. Drugs were administered using a stepwise increasing intravenous infusion over 30 min to facilitate the assessment of a concentration dependant change in hepatic blood flow. The first 15 min, $0.6 \text{ } \mu\text{g min}^{-1}$ of octreotide or $4 \text{ } \mu\text{g min}^{-1}$ of somatostatin was infused, and in the remaining 15 min the dosing rate was doubled to $1.2 \text{ } \mu\text{g min}^{-1}$ of octreotide or $8 \text{ } \mu\text{g min}^{-1}$ of somatostatin. A third placebo treatment was included consisting of a 30 min stepwise infusion with saline.

Because of the reported two-compartment pharmacokinetic properties of sorbitol, the constant rate infusion of sorbitol ($0.05 \text{ g per min}^{-1}$ over 170 min.) used during hepatic blood flow assessment was preceded by a 2 g loading bolus dose. A surrogate for total hepatic blood flow was determined by measuring flow in the right portal vein using echo-Doppler as described previously [10]. The right portal vein receives only a part of total hepatic flow and although strong indications exist that right portal vein flow can be used to assess relative changes in flow, absolute hepatic flow cannot be accurately determined.

Sorbitol requires a two-compartment open model to describe its kinetics. This may be implemented using the following set of differential equations:

$$\frac{dC_{1t}}{dt} = \frac{R_{\text{inf}}}{V_1} - \left(\frac{CL}{V_1} + k_{12} \right) C_{1t} + k_{21} C_{2t} \quad (7a)$$

$$\frac{dC_{2t}}{dt} = k_{12} C_{1t} - k_{21} C_{2t} \quad (7b)$$

C_{1t} and C_{2t} are the concentrations in the central and

peripheral compartments, V_1 is the volume of the central compartment and k_{12} and k_{21} are the micro rate constants associated with transport between the two compartments.

Solving the differential equations requires an expression for the clearance and therefore for the flow for all possible time points, and not only for the points that were actually measured. Because sorbitol requires a set of two differential equations, an essentially non-parametric expression for clearance at each time point similar to equation (6) for ICG cannot be obtained. Unfortunately, somatostatin and octreotide concentrations could not be accurately determined thereby ruling out direct (somatostatin/octreotide)concentration-(hepatic blood flow)effect modelling. Initially a model was entertained where sorbitol clearance was assumed to be a linear function of somatostatin or octreotide concentration. Since these concentrations were unavailable, a simple one-compartment pharmacokinetic model was assumed where the associated half-life was yet another parameter to be estimated. With this model, no half-life estimates for somatostatin or octreotide could be obtained leading to adequate prediction of the sorbitol profile. Inspection of the echo-Doppler results provided a clue to the explanation for this discrepancy. With the previous assumptions, the decrease must be the same for each step. The Doppler data indicate however a rapid decrease in flow during the first step of the intervention and only a minor additional decrease for the second step. Additionally, the decrease in flow is similar for somatostatin and octreotide, but return to basal conditions is much slower for octreotide than for somatostatin. These two findings are in contradiction with the assumption of a linear concentration-effect relationship or the assumption of linear and one-compartment pharmacokinetics for somatostatin and octreotide, or both.

A pragmatic alternative to describe the flow-profile was required. Inspired by the shape of the Doppler flow profile, the shape of the initial drop in flow was modelled using a 30 min constant rate infusion of substance I (i.e. not the two-step graded infusion of the actual intervention) to a maximum of 1 with a half-life of $t_{1/2\text{drop}}$:

$$I_t = 1 - e^{-\left(t - t_{\text{start}}\right) \frac{\ln 2}{t_{1/2\text{drop}}}} \quad (8a)$$

To account for the distinct difference in onset and subsequent disappearance of effect, I was allowed to disappear after the end of the infusion with a different half-life ($t_{1/2\text{rise}}$):

$$I_t = I_{t_{\text{stop}}} \cdot e^{-\left(t - t_{\text{stop}}\right) \frac{\ln 2}{t_{1/2\text{rise}}}} \quad (8b)$$

The entire sorbitol clearance profile with I equal to zero prior to the intervention was described using:

$$CL_t = CL_{\text{basal}} - CL_{\text{max}} \cdot I_t \quad (8c)$$

All sorbitol profiles for the somatostatin and octreotide occasions were analysed using the set of equations resulting from combining (7a), (7b), (8a), (8b) and (8c), resulting in estimates for parameters V_1 , k_{12} , k_{21} , $t_{1/2\text{drop}}$, $t_{1/2\text{rise}}$, CL_{basal} and CL_{max} . Individual curves were analysed using the NONMEM software program (NONMEM Version IV, NONMEM Project Group, University of California, San Francisco, CA) which, although intended for population pharmacokinetics, can be configured to provide individual ordinary least squares estimates. An additive (constant

variance) residual error model was used; all syntax is available from the authors upon request.

Additionally, the curves were analysed collectively using nonlinear mixed effect modelling implemented in the NONMEM software program (using first order conditional estimation). The NONMEM methodology provides estimates of mean and inter-individual variability of the population parameters, which may then be used to obtain empirical Bayes estimates for each individual and treatment. These estimates are a weighted combination of the information from the individual and the overall population information. Parameter estimates for individual curves calculated using ordinary individual least squares estimation, may depend on only one or two points and if these are ill placed ('outliers'), implausible estimates can result. By combining all available information from all subjects, the influence of these points is reduced, resulting in more reproducible individual estimates [11]. The method also allows formal comparison using the likelihood-ratio test of different (nested) models providing inference statements for possible differences in parameter values between treatments.

Hepatic blood flow was calculated using the predicted sorbitol clearance profile in combination with equation (2), assuming that hepatic clearance of sorbitol is 90% of total clearance [4], the extraction ratio is 0.96 (at a flow of 1.5 l min^{-1}) and the haematocrit is 0.42. The well-stirred model and the parallel tube model give essentially the same translation of clearance into flow. If flow had been increased from basal values instead of decreased, this might not have been the case. The relationship between calculated hepatic blood flow and echo-Doppler measured flow in the right portal vein was investigated using conventional linear regression.

Results

Parameters for individual ordinary least squares descriptions of the sorbitol profile for either treatment are presented in Table 2a and 2b. These same tables present the results from the NONMEM analysis and the resulting individual empirical Bayes estimates. Using these empirical Bayes estimates, predicted profiles were generated. Sorbitol concentration measurements (mean $\pm$ s.d.) and predictions (mean) against time are presented in Figure 4. Sorbitol clearance predictions (mean $\pm$ s.d.) and echo-Doppler flow measurements (mean $\pm$ s.d.) against time are presented in Figure 5. Note that although the global shape of the clearance profile was inspired by the echo-Doppler data, the ultimate shape and parameter estimates are determined by the sorbitol profile alone. Parameters for the regression of predicted hepatic blood flow on echo-Doppler measured right portal vein flow are presented in Table 3.

The sorbitol profile for the placebo treatment could not be described using a one-compartment model but was adequately described by a two-compartment model (Figure 4).

Several of the estimates obtained using individual ordinary least squares are clearly deviant and mean parameter values cannot generally be used due to severe outliers. This instability is greatly reduced for the empirical Bayes estimates resulting from the NONMEM analysis. The final

Table 2a Parameters for example 2: Sorbitol during infusion of octreotide.

	Mean	s.d.	Min	Max
<i>Individual least squares estimates</i>				
$t_{1/2\text{drop}}$ (min)	0.62	0.83	0.13	2.28
$t_{1/2\text{rise}}$ (min)	195	217	29	624
CL_{basal} (l min^{-1})	0.930	0.232	0.696	1.240
CL_{max} (l min^{-1})	0.283	0.098	0.130	0.418
<i>NONMEM estimates</i>				
$t_{1/2\text{drop}}$ (min)	0.19	0.00		
$t_{1/2\text{rise}}$ (min)	126	104		
CL_{basal} (l min^{-1})	0.920	0.148		
CL_{max} (l min^{-1})	0.256	0.063		
<i>Empirical Bayes estimates</i>				
$t_{1/2\text{drop}}$ (min)	0.19	0.00	0.19	0.19
$t_{1/2\text{rise}}$ (min)	137	85	64	295
CL_{basal} (l min^{-1})	0.964	0.184	0.763	1.225
CL_{max} (l min^{-1})	0.276	0.065	0.175	0.358

$t_{1/2\text{drop}}$: half-life for flow decrease during intervention; $t_{1/2\text{rise}}$: half-life for return to basal situation; CL_{basal} : basal plasma clearance; CL_{max} : maximal drop in plasma clearance.

Table 2b. Parameters for example 2: Sorbitol during infusion of somatostatin

	Mean	s.d.	Min	Max
<i>Individual least squares estimates</i>				
$t_{1/2\text{drop}}$ (min)	31.3	76.0	0.08	186.5
$t_{1/2\text{rise}}$ (min)	24.7	36.8	0.11	78.4
CL_{basal} (l min^{-1})	0.926	0.174	0.738	1.213
CL_{max} (l min^{-1})	0.819	1.302	0.268	3.476
<i>NONMEM estimates</i>				
$t_{1/2\text{drop}}$ (min)	0.19	0.00		
$t_{1/2\text{rise}}$ (min)	4.29	3.55		
CL_{basal} (l min^{-1})	0.920	0.148		
CL_{max} (l min^{-1})	0.256	0.063		
<i>Empirical Bayes estimates</i>				
$t_{1/2\text{drop}}$ (min)	0.19	0.00	0.19	0.196
$t_{1/2\text{rise}}$ (min)	6.37	6.78	2.24	20.02
CL_{basal} (l min^{-1})	0.906	0.139	0.742	1.096
CL_{max} (l min^{-1})	0.259	0.047	0.192	0.328

$t_{1/2\text{drop}}$: half-life for flow decrease during intervention; $t_{1/2\text{rise}}$: half-life for return to basal situation; CL_{basal} : basal plasma clearance; CL_{max} : maximal drop in plasma clearance.

NONMEM model presented here, estimates identical population parameters for the two treatments except for the $t_{1/2\text{rise}}$ estimate. This choice is the result of comparing different models where parameters are allowed to differ between treatments. None of the alternative model resulted in a significant improvement in fit as judged by the associated likelihood-ratio tests. This means that somatostatin and octreotide both reach the maximal effect almost immediately with a comparable decrease in clearance of $0.26 \pm 0.06 \text{ l min}^{-1}$ from $0.92 \pm 0.15 \text{ l min}^{-1}$. This translates into a lowering of hepatic blood flow from 1.48 l min^{-1} to 1.07 l min^{-1} . Return to baseline however, is much slower for octreotide than for somatostatin. Estimated half-lives are $126 \pm 104 \text{ min}$ and $4.29 \pm 3.55 \text{ min}$ respectively.

Figure 4 Observed (mean $\pm$ s.d.) and predicted (mean) profiles of sorbitol plasma concentration for the octreotide (●) and somatostatin (○) and placebo (□) treatment. **Figure 5** Top panel: predicted (mean $\pm$ s.d.) profiles of sorbitol plasma clearance for the octreotide (●) and somatostatin (○) treatment. Bottom panel: observed (mean $\pm$ s.d.) echo-Doppler measured flow in the right portal vein for the octreotide (●) and somatostatin (○) treatment.**Table 3** Parameters for example 2: Predicted hepatic blood flow versus measured echo-Doppler right portal vein flow.

	Mean	s.d.	Min	Max
<i>Octreotide</i>				
slope	2.06	0.89	0.89	3.34
intercept (l min^{-1})	0.538	0.202	0.296	0.884
<i>Somatostatin</i>				
slope	2.03	0.85	0.92	2.98
intercept (l min^{-1})	0.585	0.091	0.486	0.735

ively. The data provided insufficient information for the NONMEM methodology to estimate inter-individual variability in the half-life for flow-drop during the intervention ($t_{1/2\text{drop}}$). This causes the empirical Bayes estimates for all subjects to be identical. Although this seems unlikely, it simply means that the predicted profile does not improve if different values are estimated for the different individuals.

The predicted sorbitol clearance profile shows some correspondence to the measured echo-Doppler flows. Regression of predicted hepatic blood flow onto measured right portal vein flow indicates that the intercept is non-

zero ($0.56 \pm 0.15 \text{ l min}^{-1}$). This may indicate that there is a constant amount of flow unaffected by the interventions which could be attributed to hepatic arterial flow. Slope-estimates have approximate three-fold inter-individual variability corroborating the impression that echo-Doppler measurements cannot be used to obtain a reliable estimate of absolute total hepatic blood flow. Changes in flow however, seem to be adequately reflected.

Discussion

The most complex part in this analysis is obtaining a continuous description of the clearance/flow profile that will result in satisfactory prediction of sorbitol concentration. Relatively simple approaches would be measuring the intervening drug and estimating the concentration effect relationship, or linear interpolation in the echo-Doppler flow profile assuming this to be a constant fraction of total hepatic flow. However, somatostatin and octreotide concentrations could not be adequately determined eliminating PK/PD modelling as an option. The first Doppler measurement during the intervention is at the end of the first step and therefore contains no information on the actual flow profile during that first step. Linear interpolation in the Doppler profile, resulted in flow-predictions changing too slowly to predict the acute rise in sorbitol concentrations immediately following the intervention. We therefore had to resort to a somewhat artificial representation, which nevertheless seems to capture the essence of the experimental results. In order to obtain an adequate sorbitol concentration profile, an immediate drop in clearance is required with gradual recovery to the basal situation. This is substantiated by the echo-Doppler measurements. The fact that the onset of effect has a much shorter half-life than the return to basal conditions is an indication of nonlinearity in either the kinetics of the intervening drugs or of the concentration-effect relationship (or both).

All modelling exercises so far have assumed that intrinsic clearance of the marker compound is unaffected by the interventions and that changes in marker concentration are attributable to changes in flow only. Echo-Doppler profiles indicate that changes in flow are actually present and for the ICG/exercise example, we may assume on the basis of physiology that flow changes do actually occur. Nevertheless, if intrinsic clearance is affected, this will influence the adequacy of the predictions for hepatic blood flow. Given the high intrinsic clearance for sorbitol, even large modifications would probably not result in major changes in extraction ratio, although this does depend on which model is considered most accurate. Halving the intrinsic clearance results in a change in extraction ratio from 0.96 to 0.92 for the well-stirred model but for the parallel tube model the extraction ratio drops to 0.80. The range of possible extraction ratios for ICG is much wider and the choice of model has greater consequences. This means that ICG cannot be used to provide reliable point estimates of hepatic blood flow without direct measurement of the extraction ratio. Description of changes in flow and their relative magnitude are far less affected though, and if interest lies here, ICG should not be dismissed as a useful marker compound.

Conclusions

The concentration profiles that result from infusion of a high clearance drug can be translated into corresponding clearance and flow profiles. This is much easier for a drug with one-compartment kinetics like ICG than for sorbitol that requires a two-compartment model; postulation of a parametric model for the flow profile is required in this case.

The high extraction ratio for sorbitol allows easier translation from clearance to flow than for ICG, and is probably less subject to inter-individual variability. This may favour the use of sorbitol in steady state conditions for the accurate determination of hepatic blood flow. Dynamic situations, where interest lies mostly in the profile and relative magnitude of flow-changes, may favour the use of ICG.

We would like to thank Dr Paul Soons for the use of his ICG data.

References

- 1 Caesar J, Shaldon S, Chiandussi L, Guevara L, Sherlock S. The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. *Clin Sci* 1996; **21**: 43–57.
- 2 Soons PA, De Boer A, Cohen AF, Breimer DD. Assessment of hepatic blood flow in healthy subjects by continuous infusion of indocyanine green. *Br J Clin Pharmacol* 1991; **32**: 697–704.
- 3 Pang KS, Rowland M. Hepatic clearance of drugs. I. Theoretical considerations of a 'well-stirred' model and a 'parallel tube' model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J Pharmacokinet Biopharm* 1977; **5**: 625–653.
- 4 Morgan DJ, Smallwood RA. Clinical significance of pharmacokinetic models of hepatic elimination. *Clin Pharmacokinet* 1990; **18**: 61–76.
- 5 Zeesh J, Lange H, Bosch J, *et al.* Steady-state extrarenal sorbitol clearance as a measure of hepatic plasma flow. *Gastroenterology* 1988; **95**: 749–759.
- 6 Daneshmend TK, Jackson L, Roberts CJC. Physiological and pharmacological variability in estimated hepatic blood flow in man. *Br J Clin Pharmacol* 1981; **11**: 491–496.
- 7 Sonnenberg GE, Keller U, Perruchoud A, Burckhardt D, Gyr K. Effect of somatostatin on splanchnic haemodynamics in patients with cirrhosis of the liver and in normal subjects. *Gastroenterology* 1981; **80**: 526–532.
- 8 Wahren J, Efendic S, Luft R, Hagenfeldt L, Björkman O, Felig P. Influence of somatostatin on splanchnic glucose output metabolism in postabsorptive and 60-hour fasted humans. *J Clin Invest* 1977; **59**: 299–307.
- 9 Wahren J, Eriksson LS. The influence of the long-acting somatostatin analogue on splanchnic haemodynamics and metabolism in healthy subjects and patients with liver cirrhosis. *Scand J Gastroenterol* 1986; **21** (Suppl 119): 103–108.
- 10 Burggraaf J, Schoemaker RC, Kroon JM, Huisman LGM, Kluft C, Cohen AF. The influence of dDAVP on endogenous fibrinolysis, haemodynamics and liver blood flow in healthy subjects. *Clin Sci* 1994; **86**: 497–503.
- 11 Schoemaker RC, Cohen AF. Estimating impossible curves using NONMEM. *Br J Clin Pharmacol* 1996; **42**: 283–290.

(Received 23 June 1997,
accepted 6 January 1998)