J. Pharm. Pharmacol. 1985, 37: 471–475
Received November 26, 1984

Influence of serum protein binding on hepatic clearance of *S*-disopyramide in the rabbit

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The effect of changing the protein binding of *S*-disopyramide, a compound that is highly extracted by the liver on its hepatic clearance in rabbits is described. Using indocyanine green clearance as a means of measuring hepatic blood flow, qualitative changes were observed which are in agreement with the results predicted by both the 'parallel tube' and the 'well-stirred' models. However, neither model alone could explain the quantitative changes. Suggestions are put forward to explain these results.

The effects of the plasma protein binding changes on a compound that is highly extracted by liver have been examined in the rat isolated perfused liver over a wide range of protein binding values by Rowland et al (1983, 1984). The observations strongly support the accepted kinetic theories of the relations of protein binding to clearance and are reported to correspond better to the 'parallel tube' model than the 'well-stirred' model (Pang & Rowland 1977), although the differentiation between the two flow models may not be of significance in-vivo since the variation in plasma protein binding in-vivo rarely encompasses the large range used. Also, as the models relate to the unbound fraction in *blood* rather than the unbound fraction in *serum*, which is the fraction usually measured, in-vivo changes in binding relate not only to serum proteins but also to red blood cells.

We have evaluated the effect of serum protein binding changes on the in-vivo clearance of disopyramide, in conditions where large changes in serum protein binding have been made.

METHODS

Four male New Zealand white rabbits, (2·55–3·25 kg) were used. Disopyramide (prepared as the *S*-enantiomer as described by Burke Jr et al 1980 and Huang & Øie 1983), as the test drug, was infused into a marginal ear vein using a precalibrated pump (Harvard Model 975). To maintain steady-state concentrations at two levels, the drug was infused at 0·027 mg min^{-1} kg^{-1} into rabbits 1 and 4 and at 0·081 mg min^{-1} kg^{-1} into rabbits 2 and 3. A loading infusion, approximately 3·8 times the maintenance infusion rate, was given over 15 min to achieve the desired steady-state concentration. During the steady-state infusion, doses of 13, 27 and 40 mg kg^{-1} human glycoprotein fraction VI in sterile water were injected at 80, 160 and 240 min, respectively. Blood samples were taken before and at 40, 50, 60, 70, 120, 130, 140, 150, 200, 210, 220, 230, 280, 290, 300 and 310 min after the start of drug administration. At 40, 60, 120, 140, 200, 220, 280 and 300 min, 1 ml blood was taken and frozen for determination of disopyramide concentration in whole blood. At all other times, 3 ml blood was withdrawn, 1 ml was frozen for drug assay and the remainder centrifuged and the serum taken for determination of total and unbound disopyramide. The hematocrit value was determined for each blood sample. Urine was collected throughout and the amount of disopyramide excreted unchanged was determined.

Measurement of indocyanine green clearance

Indocyanine green (ICG) clearance was determined at each human glycoprotein fraction VI level using the following method: A 0·1 mg kg^{-1} dose of ICG was dissolved in 0·3 ml sterile water and injected into a marginal ear vein in the rabbit within 3 s. Disopyramide infusion was temporarily stopped during the injection of dye. Blood was collected from the marginal ear vein in the other ear by continuous withdrawal using a Harvard infusion/withdrawal pump (model 931) at a rate of 0·36 ml min^{-1} over 8 min. Plasma was separated by centrifugation at 300*g* for 10 min. The hematocrit (H) was determined and dye concentration measured. The blood clearance of ICG ($Cl_{B,ICG}$) was then calculated using the following equation:

* Correspondence.

$$Cl_{B,ICG} = \frac{\text{dose}}{\bar{C}_{p,ICG}.\tau.(1-H)} \quad (1)$$

where τ is the withdrawal time and $\bar{C}_{p,ICG}$ is the plasma concentration in the sample (averaged plasma concentration over the total collection period).

Assay methods

Disopyramide was assayed by a high performance liquid chromatographic method (Huang & Øie 1983). ICG was also assayed by HPLC. 100 µl acetonitrile was added to 100 µl serum, mixed and centrifuged at 7000*g* for 2 min. 50 µl of the supernatant was injected onto a C18 10 µm column (25 cm length 4·6 mm base, Alltech Assoc. Deerfield IL). The samples were eluted with acetonitrile, tetrabutylammonium hydrogen sulphate, 85% phosphoric acid and 5 M sodium hydroxide (50:50:0·05:0·05:0·12) at a flow rate of 1 ml min^{-1}. The detection wavelength was 800 nm, and detection limit (7:1 peak-to-noise ratio) 0·5 ng in the injected sample.

Data analysis. The total body clearance (Cl_{TB}) of disopyramide was calculated from the steady-state blood concentration ($C_{B,ss}$ = average concentration at each level) using the relation: $Cl_{TB} = R/C_{B,ss}$ where R is the disopyramide dosing rate. The fraction of drug excreted in urine unchanged (fe) was determined by the amount of drug recovered in the urine sample divided by the amount administered during each steady-state period. The non-renal clearance, which is assumed to be hepatic clearance (Cl_{HB}), was then calculated using $Cl_{HB} = Cl_{TB}.(1 - fe)$. The unbound fraction of disopyramide (fu), measured by equilibrium dialysis, (Huang & Øie 1983) was corrected for volume shift and concentration-dependence using a method similar to that described by Tozer et al (1983): assuming there is only one class of binding sites and that the binding follows the law of mass action, the concentration of binding sites (Pt) inside the dialysis cell at equilibrium was calculated using an equation rearranged from the law of mass action:

$$Pt = (Kd + Cu).[(1/fu) - 1] \quad (2)$$

where Cu is the disopyramide concentration measured in the buffer side after equilibrium dialysis, and Kd is the dissociation constant of disopyramide-α_1-acid glycoprotein complex in rabbit serum. The value of Kd was assumed to be 4·7 µM (Huang & Øie 1983). Because there was consistent 10% water flux from the buffer side to the serum side in the equilibrium dialysis cells with the dialysis procedure used, the binding-site concentration (Pt*) pre-dialysis was: Pt* = Pt × 1·1. The unbound drug concentration in serum (Cu*) corrected for volume shift and concentration-dependence was then calculated by another rearrangement of the law of mass action

$$Cu^* = \frac{Cp - Kd - Pt^* + \sqrt{(Cp - Kd - Pt^*)^2 + 4.Cp.Kd}}{2} \quad (3)$$

where Cp is the total disopyramide concentration measured in serum before equilibrium dialysis. The corrected unbound fraction of drug in serum (fu*) was calculated by $fu^* = Cu^*/Cp$ and unbound fraction of disopyramide in blood (fu_B^*) was obtained from $fu_B^* = Cu^*/C_B$.

The observed hepatic blood clearance was compared with the theoretically predicted values using the 'well-stirred' model and 'parallel tube' model. The hepatic blood flow (Q_H) was obtained from the ICG clearance assuming an extraction ratio of dye of 0·9 (Guentert & Øie 1980). The intrinsic clearance of disopyramide (Cl_I) was calculated using the equations for the well-stirred model

$$Cl_{HB} = \frac{Q_H.fu_B^*.Cl_I}{Q_H + fu_B^*.Cl_I} \quad (4)$$

and for the parallel tube model

$$Cl_{HB} = Q_H[1 - efu_B^* Cl_I/Q_H] \quad (5)$$

and the clearance determined before glycoprotein injection. The intrinsic clearance was assumed to be constant throughout the study in each rabbit. Equations 1, 4 and 5 were subsequently used to calculate the predicted hepatic blood clearance based upon the unbound fraction obtained and the measured hepatic blood flow.

RESULTS

The corrected unbound fraction, unbound drug concentration, observed hepatic blood clearances, and the average ICG clearances are in Table 1. The corrected unbound fraction did not deviate by more than 10% from the uncorrected values (fu*/fu ranged from 0·88–1·00). Because a water flux from buffer to plasma tends to increase the unbound fraction during equilibrium dialysis, and in a condition where there is saturation of the binding site, the unbound fraction tends to be underestimated, the errors due to dialysis usually correct each other. This phenomenon is particularly true when the unbound fraction is not substantially different from the dissociation equilibrium constant of the drug-protein complex. The dye

Table 1. Observed pharmacokinetic parameters of disopyramide and clearance of indocyanine green in rabbits treated with human glycoprotein fraction VI.

Rabbit	Study period (min)	fu*	fu^*_B	Cu* (mg litre^{-1})	Cl_{HB} (ml min^{-1} kg^{-1})	$Cl_{B,ICG}$† (ml min^{-1} kg^{-1})
1	0–80	0·91	0·91	0·43	59	60 ± 7·2
	80–160	0·29	0·39	0·32	35	
	160–240	0·18	0·26	0·23	28	
	240–320	0·15	0·22	0·22	26	
2	0–80	0·79	0·78	1·4	46	50 ± 8·8
	80–160	0·40	0·47	1·1	37	
	160–240	0·22	0·29	0·64	33	
	240–320	0·12	0·17	0·45	30	
3	0–80	0·80	0·77	1·6	44	63 ± 10
	80–160	0·51	0·58	1·5	29	
	160–240	0·39	0·48	2·1	18	
	240–320	0·25	0·34	1·9	13	
4	0–80	0·80	0·86	0·43	48	45 ± 3·9
	80–160	0·29	0·39	0·25	39	
	160–240	0·13	0·19	0·14	39	
	240–320	0·066	0·10	0·092	30	

† Mean ± s.d. for each rabbit studied.

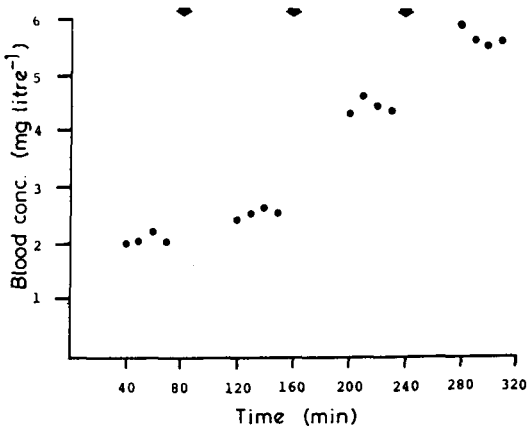

FIG. 1. Blood concentration of disopyramide with time in rabbit no. 3. The arrows indicate injection time of α-1-acid glycoprotein. The infusion rate of disopyramide was kept constant throughout the experiment at 0·081 mg min^{-1} kg^{-1}.

clearance values were similar to values obtained in earlier studies in rabbit (Guentert & Øie 1980).

The whole blood concentrations obtained for a representative animal are in Fig. 1. There was no trend of increasing or decreasing concentrations at any phase of the studies and the values were considered to be steady state levels and averaged. The coefficient of variation at each level was mean 0·09 and range 0·03–016.

The relations between the hepatic clearance and the unbound fraction of disopyramide in blood is plotted in Fig. 2 and compared with the theoretically predicted values based on the 'well-stirred' and 'parallel tube' models.

The fraction of drug excreted unchanged in the urine varied during each study and with a range of 9·6–18·9% in rabbit no. 1, 10·9–21·1% in rabbit no. 2, 13·5–26·1% in rabbit no. 3 and 8·1–16% in rabbit no. 4. There was no tendency for the values to change with human glycoprotein factor VI administration.

It is apparent that the hepatic blood clearance decreased with the decrease of unbound disopyramide fraction after injection of human glycoprotein fraction VI. In general, the observed relation is qualitatively consistent with the proposed models; however, no distinction between the 'well-stirred' and 'parallel tube' models can be concluded.

DISCUSSION

As the hepatic blood flow, measured as ICG clearance, did not change throughout the studies, the results suggest that when protein binding of disopyramide which is highly extracted by the liver is changed sufficiently in-vivo, the total clearance decreases and the drug no longer acts as a compound with a true high extraction ratio.

Although the changes can be qualitatively predicted from either the 'well-stirred' or 'parallel tube' model, the observed changes do not allow for any discrimination between the models. Part of the explanation for this inability to discriminate could be due to the inaccuracy in the hepatic blood flow determinations. In rabbits the ICG clearance is 80–100% of the hepatic blood flow (Guentert & Øie 1980). An average of 90% was used for these calculations. Because the reference hepatic clearance of disopyramide (no human glycoprotein frac-

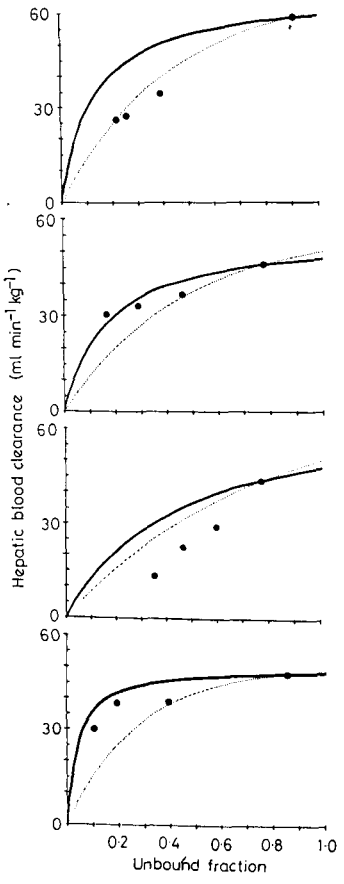

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FIG. 2. Relation between hepatic blood clearance and protein binding in blood of four rabbits treated with human glycoprotein fraction VI (· · · ·) predicted values for the 'parallel tube' model and (——) predicted values from the 'well-stirred' model with linear binding during the extraction process using the data with no human glycoprotein fraction VI added as the reference point. (●) observed data.

is generally not very different, therefore a small error in any value may create large errors in the estimated relations between the clearance and unbound fraction of drug for the different models.

Another problem is that disopyramide exhibits saturable binding to α_1-acid-glycoprotein in the concentration range tested (Huang & Øie 1983). This causes a change in binding as the drug is extracted in the liver (Huang & Øie 1984); the clearance tends to be overestimated when such binding changes are not taken into consideration. However, because of the low unbound concentration of the drug in relation to the association binding constant found (0·06–1·3) and a blood-to-plasma ratio of approximately 1, the effect was minimal. The changes in estimated clearance were found to be <5% at all measured steady state concentrations when the binding saturability was taken into account.

The two proposed models are not truly physiological. The sinusoid can be considered neither as a well-stirred nor a parallel tube model. Because the hepatic arterial blood supply enters the sinusoid at several points along its channel, drug is introduced at several locations and, in addition, turbulence and mixing occur because of the shape of the sinusoids. These phenomena probably place the sinusoid behaviour between that of the two models. In isolated perfused livers, the arterial blood supply is eliminated, a situation closer to that of the parallel tube model. In addition, although it is apparent that all hepatocytes have the same enzyme systems available—there is evidence that the concentration of the various enzymes varies throughout the sinusoid (Pang et al 1982) and this will also cause deviation from the parallel tube model. It would appear that when the portal (and hepatic) blood flow is low and the arterial blood flow is a major contributor to the hepatic blood flow, that significant mixing of blood occurs and less drug enters at the anterior of the sinusoid. Then the hepatic elimination might be expected to deviate from the parallel tube model towards the well stirred model. As the portal blood flow is increased, results nearer those redicted by the parallel tube model will be btained. In addition, Ahmad et al (1984) found that ıe extraction ratio for drug entering the hepatic tery to be smaller than that for drug entering the ɔrtal vein.

The two animals with the lowest hepatic blood ɔw (lowest ICG—clearance) were also those whose .ta showed the closest resemblance to the well-rred model. These results suggest that refinement of the models may be worth pursuing.

Acknowledgement

Supported in part by grant GM 28423 from the National Institutes of General Medical Sciences, USA.

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