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A STUDY OF FACTORS INFLUENCING DRUG DISPOSITION IN CHRONIC LIVER DISEASE, USING THE MODEL DRUG (+)-PROPRANOLOL

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1 The pharmacokinetics, following i.v. administration of (+)-propranolol (40 mg) have been compared to *in vitro* measurement of protein binding and biochemical parameters of liver function in six normal subjects and twenty patients with stable chronic liver disease.

2 The clearance of (+)-propranolol decreased with evidence of increasing severity of impairment of liver function correlating significantly with a fall in serum albumin, a rise in bilirubin and a prolongation in prothrombin index.

3 The clearance of (+)-propranolol correlated with and was numerically similar to the clearance of indocyanine green in normal subjects and also in patients with chronic liver disease.

4 Protein binding was decreased in chronic liver disease, but this change was not related to changes in plasma proteins.

5 In normal subjects and patients without ascites the volume of distribution increased with decreases in protein binding.

6 Ascites was associated with a further increase in the volume of distribution.

7 The considerable variation in half-life largely depends on changes in liver blood flow, the degree of protein binding and the plasma protein pool size.

Introduction

Liver disease has the potential of influencing many independent aspects of drug disposition. As the mechanisms involved and the degree of variation in drug disposition can only be assessed by direct measurement of drug concentrations in patients with liver disease, it is not surprising that there have been relatively few studies providing information on the possible factors involved. Of the studies, most have been based on the measurement of half-life ($T_{\frac{1}{2}}$) of the drug under investigation. These studies have failed to differentiate changes in either the distribution of the drug within the body or the efficiency of the elimination process i.e. clearance; these are the two independent variables upon which the $T_{\frac{1}{2}}$ is dependent. Drug distribution is dependent on several factors amongst which are regional blood flow, protein, blood and tissue binding and the mass of tissue. Drug clearance is dependent on the sum of the clearances of the various routes of elimination. For a drug metabolized by the liver, the hepatic clearance will depend on the amount and activity of the drug metabolizing enzymes. Furthermore, if the clearance is high then the rate of delivery of the drug to the liver by the liver blood flow will become a rate-limiting factor so that the clearance will approach liver blood flow and the hepatic extraction ratio approximate to unity (Rowlands, Benet & Graham, 1973).

Propranolol, which has extensive tissue distribution and high plasma protein binding (Evans & Shand, 1973) is rapidly metabolized by the liver so that its hepatic clearance is high, approximating to the expected liver blood flow in normal subjects (Evans, Nies & Shand, 1973). However, in the rhesus monkey the reduction in liver blood flow due to β-adrenergic receptor blockade by racemic mixture of propranolol is associated with a proportionate reduction in its own clearance when compared to the clearance of the vasoinactive isomer (+)-propranolol (Nies, Evans & Shand, 1973). Similar reductions in the clearance of racemic mixture of propranolol compared to (+)-propranolol have been reported in man (George, Fenyvesi, Conolly & Dollery, 1972). In order to avoid this self-induced haemodynamic drug interaction, the vasoinactive isomer (+)-propranolol which is metabolized in a similar manner to racemic mixture of propranolol has been used as a model drug in an investigation of

the influence of chronic liver disease on the distribution, clearance and therefore the $T_{½}$ of this drug.

Methods

Six normal subjects not receiving drugs were studied as controls, individual studies being spread over the entire period of investigation. Twenty patients with histologically proven stable chronic liver disease consented to be investigated. Routine clinical examination and biochemical liver function tests were followed in appropriate patients by barium swallow and oesophagoscopy to exclude the presence of oesophageal varices. If the prothrombin index was initially prolonged, parenteral Vit K was administered prior to the study. A full drug history was taken, all drugs being maintained unchanged throughout the test period. Nitrazepam was the only hypnotic administered. Patients with acute liver disease, evidence of biliary obstruction or patients receiving drugs known to induce the hepatic microsomal enzymes were excluded.

Indocyanine green (ICG, 0.5 mg/kg body weight) was administered as a rapid i.v. bolus, after withdrawal of blood (30 ml) for blank, standard curves and protein binding studies. Peripheral venous samples were obtained through an indwelling needle at 3 min intervals for 21 min in normal subjects, and at wider time intervals up to 90 min in patients with severe chronic liver disease. Plasma concentrations were measured by the method of Caesar, Sheldon, Chiandussi, Guevara & Sherlock (1961) within 2 h of drug administration. Linear calibration curves were obtained from dilutions of a sample of the ICG solution which had been administered to the individual subject in blank plasma of that subject.

Immediately after the final ICG sample had been withdrawn an i.v. bolus of (+)-propranolol (40 mg) was administered over 5 minutes. An average of ten 2 ml blood samples were taken over a period of 12 h in control subjects and up to 48 h in severe chronic liver disease. Whole blood samples were assayed by the method of Shand, Nuckolls & Oates (1970). Plasma protein binding of (+)-propranolol was measured by equilibrium dialysis over a 20 h dialysis with an approximate plasma concentration of 133 ng/ml similar to the method of Evans & Shand (1970).

Calculations

The clearance of (+)-propranolol was estimated from the area under the whole blood concentration-time curve extrapolated to infinity (AUC) as:

$$\text{clearance} = \frac{\text{dose}}{\text{AUC}} \quad (1)$$

The rate of elimination of the late phase (β) was estimated from least squares regression analysis and expressed as the half-life. The apparent volume of distribution ($V_D\beta$) was calculated (Gibaldi, Nagashima & Levy, 1969) as:

$$V_D\beta = \frac{\text{clearance}}{\beta} \quad (2)$$

Protein binding was estimated as:

Protein binding =

$$= \frac{\text{concentration in plasma} - \text{concentration in buffer}}{\text{concentration in plasma}} \times 100\% \quad (3)$$

In normal subjects the plasma curve of ICG is monoexponential with a high hepatic extraction ratio (Caesar *et al.*, 1961) so that its clearance approaches and is limited by liver blood flow. In patients with severe chronic liver disease however, the plasma decay curve becomes biexponential after 15-20 min indicating the appearance of a second rate-limiting factor, and conforms with the exponential function (Reigelman, Loo & Rowland, 1968)

$$Cp_{(t)} = Ae^{-\alpha t} + Be^{-\beta t} \quad (4)$$

where $Cp_{(t)}$ is the plasma concentration at time t; A and B are constants; α and β are the rapid and slow exponential disposition constants with appropriate $T_{½}$ (α) & $T_{½}$ (β).

It has been demonstrated that in liver disease ICG is not distributed extravascularly apart from the liver (Cherrick, Stein, Leevy & Davidson, 1960) and that there is no other route of elimination, therefore the α phase of the plasma decay curve should represent distribution of drug into the liver and be dependent on liver blood flow. The clearance of ICG has been estimated from the AUC of the initial distribution phase after correcting plasma concentrations to whole blood concentrations using the haematocrit:

$$\text{Clearance} = \frac{\text{Dose}}{(A+B)} \cdot \frac{0.693}{T_{½}(\alpha)} \quad (5)$$

Results

The age, weight and sex distribution in control subjects and patients with stable chronic liver disease are similar (Table 1). The diagnoses, clinical complications and details of (+)-pro-

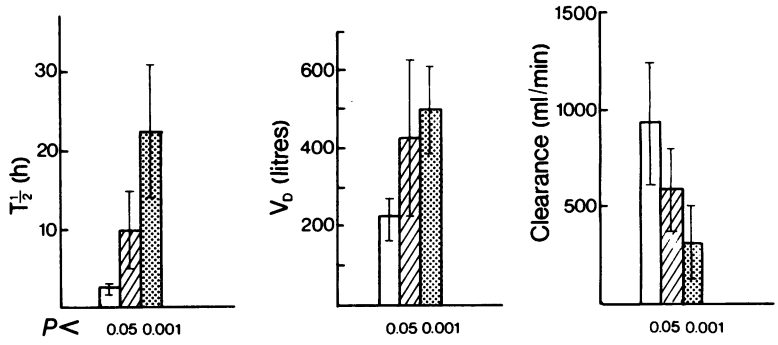

Figure 1 The mean ± s.d. $T_{½}$ volume of distribution (V_D) and clearance following the i.v. administration of (+)-propranolol (40 mg). □ normal controls, ▨ patients with chronic liver disease and a serum albumin above 3g/100 ml, ▩ patients with chronic liver disease and a serum albumin below 3g/100 ml. *P* values in comparison to normal controls.

Table 1 Sex, age and weight in six normal subjects and twenty patients with chronic liver disease (mean ± s.d.)

	Sex			
	M	*F*	*Age (years)*	*Weight (kg)*
Controls	4	2	54 ± 15	65 ± 8.50
Chronic liver disease	14	6	56 ± 12	66 ± 13

pranolol disposition of all subjects investigated are listed in Table 2 and include amongst the patients with chronic liver disease, a wide variation of clinical complications associated with chronic liver disease.

The arbitrary division of patients into mild or severe chronic liver disease was made by using a serum albumin concentration of 3g/100 ml as a dividing line. This demonstrated a progressive increase in the $T_{½}$ of (+)-propranolol from 2.9 ± 0.6 h in control subjects to 9.8 ± 5.1 h in patients with mild chronic liver disease to 22.7 ± 9 h in those patients with severe chronic liver disease, thus there is almost an eightfold increase in patients with severe liver disease compared to control subjects. This prolongation was associated with a twofold increase in the volume of distribution and a fourfold decrease in clearance (Figure 1). There were significant correlations between the $T_{½}$ and clearance of (+)-propranolol and the clearance of ICG, the serum albumin, the serum bilirubin and the prothrombin index, but not with other parameters. The volume of distribution only correlated with the prothrombin index (Table 3).

In normal subjects the clearance of (+)-propranolol correlated with the clearance of ICG ($r = 0.84$, $P < 0.05$), the absolute values for the two clearances being similar for each individual (Figure 2). In patients with chronic liver disease the clearance of (+)-propanolol also correlated with the clearance of ICG ($r = 0.449$, $P < 0.05$) (Figure 2) the two correlations do not significantly differ from each other and have a similar variance ratio justifying a combine correlation ($r = 0.755$, $P < 0.001$). In contrast, the $T_{½}$ of (+)-propranolol had no significant correlation with the clearance of ICG in normal subjects, and when combined with patients with chronic liver disease the $T_{½}$ of (+)-propranolol had a weaker relationship with the clearance of ICG ($r = 0.529$, $P < 0.05$) (Figure 2).

Protein binding was reduced from 87.8 ± 5.16% in normal subjects to 82.3 ± 5.7% ($P < 0.05$) in patients with chronic liver disease. This decrease in binding did not correlate with total serum protein, serum albumin concentrations or with any other biochemical parameters measured. In six controls and the fifteen patients without ascites, there was a significant correlation between the percentage of free (+)-propranolol and its volume of distribution ($r = 0.900$, $P < 0.001$). Five patients with ascites had a similar correlation ($r = 0.936$, $P < 0.01$) except that the volume of distribution has a twofold increase ($P < 0.001$) for any degree of protein binding (Figure 3). It was assumed that the free drug to tissue partition was constant between subjects and that the plasma volume of distribution of ICG was a measure of plasma protein pool size in those patients without ascites allowing the plasma protein pool to be calculated in patients with ascites using mean data. The development of ascites was associated with an

Table 2 Clinical details and pharmacokinetic parameters of (+)-propranolol for all subjects

Diagnosis	Sex	Age (years)	Weight (kg)	Porto-systemic anasto-mosis	Ascites	Previous hepatic en-cephalo-pathy	Serum albumin (g/100 ml)	Serum bilirubin (mg/100 ml)	Indocyanine green clearance (ml/min)	(+)-propranolol $T_{½}$ (h)	(+)-propranolol V_D (litres)	(+)-propranolol Clearance (ml/min)
Normal control	M	55	57	–	–	–	3.7	0.4	1324	2.7	121	512
Normal control	F	72	80	–	–	–	3.9	0.8	980	3.8	220	677
Normal control	M	57	66	–	–	–	4.5	0.6	2001	3.1	232	861
Normal control	M	61	59	–	–	–	4.0	0.7	1378	2.7	286	1206
Normal control	M	52	68	–	–	–	4.1	0.6	678	2.1	243	1326
Normal control	F	27	60	–	–	–	4.4	0.9	929	2.8	227	936
Alcoholic cirrhosis	M	68	52	–	–	–	3.7	1.1	743	4.4	183	481
Alcoholic cirrhosis	M	60	85	S	–	–	3.7	2.6	672	17	593	461
Alcoholic cirrhosis	M	64	66	V	–	–	2.9	7.0	145	20	335	191
Alcoholic cirrhosis	M	64	73	V	+	+	2.9	10.0	355	31	443	166
Alcoholic cirrhosis	F	54	43	–	–	–	2.9	2.8	226	29	476	190
Alcoholic cirrhosis	F	40	42	V	+	+	2.8	5.5	1225	12.5	666	617
Chronic active hepatitis	F	45	49	V	–	–	3.9	2.5	814	2.8	261	1086
Chronic active hepatitis	F	45	49	S	–	–	3.3	2.9	875	7.2	295	476
Chronic active hepatitis	M	21	80	S	+	+	2.8	2.6	382	28.2	557	229
Chronic active hepatitis	M	21	60	S	–	+	2.1	11.5	405	12.4	416	387
Chronic active hepatitis	M	23	66	V	–	–	3.0	1.4	515	35.4	420	137
Chronic active hepatitis	M	49	74	–	–	–	2.8	8.6	678	11.1	649	672
Cryptogenic cirrhosis	M	51	90	V	+	–	3.8	1.2	1013	14.3	787	634
Cryptogenic cirrhosis	M	61	55	V	–	–	2.9	1.8	569	4.6	276	800
Cryptogenic cirrhosis	M	47	56	–	+	+	2.8	5.6	105	24.4	557	246
Cryptogenic cirrhosis	M	61	54	S	–	–	3.1	1.8	550	8.1	374	533
Post hepatitis cirrhosis	M	66	70	V	–	–	4.5	0.4	692	13	755	671
Post hepatitis cirrhosis	M	66	70	S	–	–	3.9	1.0	548	13.6	368	312
Scleroderma	F	62	70	S	–	–	3.9	1.5	507	7.4	296	614
Nodular transformation of liver	M	30	80	S	–	–	4.1	0.8	778	15.9	494	360

V oesophageal varices; S surgical portocaval anastomosis

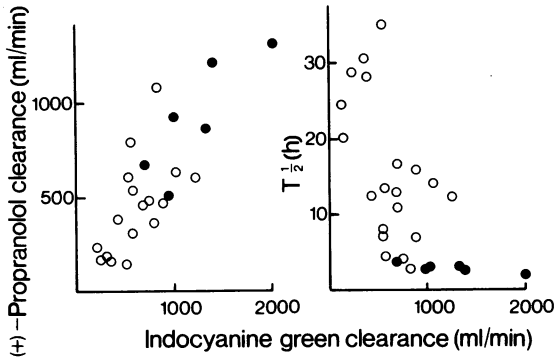

Figure 2 A comparison of the clearance and $T_{\frac{1}{2}}$ of (+)-propranolol with the clearance of indocyanine green in six normal subjects (●) and twenty patients with chronic liver disease (○), $r = 0.856$, $P < 0.001$.

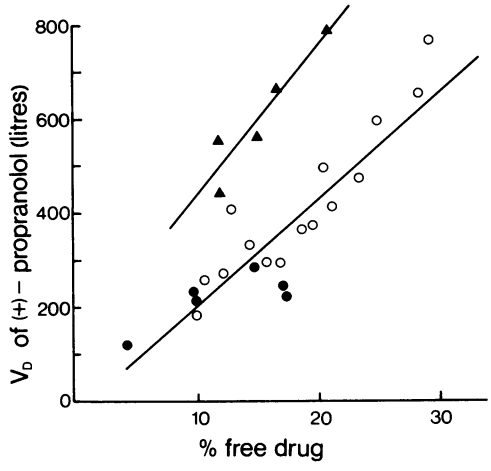

Figure 3 A comparison of the % free (+)-propranolol measured by equilibrium dialysis to the volume of distribution measured from the whole blood concentration *v.* time profile after an i.v. bolus of (+)-propranolol (40 mg) in six control subjects (●), fifteen patients with chronic liver disease (○) and five patients with chronic liver disease with ascites (▲).

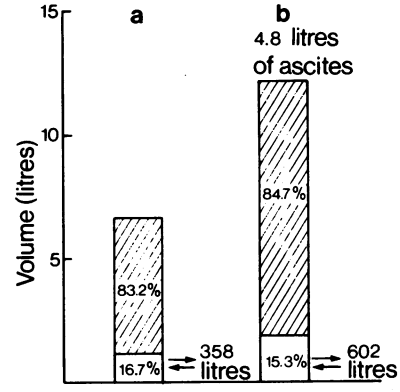

Figure 4 Mean distribution of (+)-propranolol in (a) fifteen patients with chronic liver disease without ascites and (b) five patients with ascites. Protein binding, total volume of distribution and the volume of the plasma protein pool in patients without ascites are direct measurements. The distribution of free drug to tissue is assumed to be constant. ▨ Plasma protein volume; □ free water volume.

increase of the plasma protein pool and free drug pool size without there being a change in protein binding (Figure 4).

Discussion

All the parameters of (+)-propranolol disposition following intravenous administration have been shown to be influenced by chronic liver disease. There was a threefold increase in the $T_{\frac{1}{2}}$ of (+)-propranolol in patients with chronic liver disease and a serum albumin above 3g/100 ml compared to normal subjects. This prolongation rose to nearly an eightfold increase when comparing patients with severe chronic liver disease and a serum albumin below 3g/100 ml to normal subjects (Figure 1). The correlation

Table 3 Correlation matrix comparing the pharmacokinetic parameters of (+)-propranolol to the clearance of indocyanine green and routine 'liver function tests', in six normal subjects and twenty patients with chronic liver disease

(+)-propranolol	*Indocyanine green clearance*	*Albumin*	*Prothrombin index*	*Bilirubin*	*Serum aspartate aminotransferase*	*Alkaline phosphatase*	*Globulin*
Clearance	+0.856***	+0.563**	+0.483*	−0.385*	−0.087	+0.103	−0.219
Volume of distribution	−0.215	−0.185	−0.590**	+0.268	+0.312	−0.066	+0.228
Half life	−0.621**	−0.531**	−0.578**	+0.382*	+0.114	+0.022	+0.256

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

between the $T_{½}$ of (+)-propranolol and the serum albumin and prothrombin index was the same as that previously observed with the $T_{½}$ of antipyrine (Branch, Herbert & Read, 1973). In the absence of acute hepatitis or biliary obstruction a raised bilirubin is an index of severe impairment of active biliary transport and conjugation. Thus the significant relationship between the serum bilirubin and $T½$ of (+)-propranolol further substantiates the evidence that with increasing severity of liver disease, there is an increasing effect on the disposition of (+)-propranolol. Changes in the alkaline phosphatase or serum aspartate aminotransferase did not correlate with the $T_{½}$ of (+)-propranolol in this selected group of patients, where evidence of biliary obstruction or acute hepatitis were grounds for exclusion from the study.

There is a growing literature on the influence of liver disease on drug disposition, with prolongation in the $T_{½}$ being reported for antipyrine (Branch *et al.*, 1973), phenylbutazone (Levi, Sherlock & Walker, 1968), carbenicillin (Hoffman, Cestero & Bullock, 1970), chloramphenicol (Kunin, Glazko & Finland, 1959) and rifamycin (Acocella, Bonelloa, Garimoldi, Mainardi, Tocini & Nicolis, 1972). The prolongation in $T_{½}$ observed in these studies have been attributed to liver disease causing a decrease in drug metabolizing ability. However, the $T_{½}$ is a variable which depends on the volume of distribution and the efficiency of the elimination process or clearance:

$$T_{½} = \frac{V_D \times 0.693}{\text{clearance}} \qquad (6)$$

and both these factors may be independently influenced by liver disease.

The high clearance of (+)-propranolol found in normal subjects, similar to those previously reported (Evans & Shand, 1973; Evans *et al.*, 1973), correlated with and was numerically similar to the clearance of ICG. Although one drug is metabolized and the other actively transported to the bile, their hepatic extraction ratios must have been equally high so that the clearance values represented were a slight underestimate of liver blood flow. With increasing severity of chronic liver disease the clearance of (+)-propranolol fell in proportion to the decrease in clearance of ICG. This suggests a decrease in the common rate limiting factor of the liver blood flow, either in association with a reduction of hepatic parenchymal tissue or following surgical portocaval anastomosis (Redeker, Geller & Reynolds, 1958; Redeker, Kunelis, Yamamoto & Reynolds, 1964). There was no evidence of drug metabolizing enzyme inhibition which would have been expected to cause a disproportionate decrease in the clearance of (+)-propranolol compared to the clearance of ICG.

The high binding of (+)-propranolol to plasma proteins in normal subjects was significantly reduced ($P < 0.05$) in patients with chronic liver disease; although the mean decrease was only 5%. However, the range of protein binding in patients with chronic liver disease was wide (Figure 3). The cause for the decrease is unknown as it did not correlate with the total plasma protein, or albumin concentrations, or the severity of the liver disease as assessed by the biochemical parameters measured. This is in contrast to amylobarbitone whose binding is decreased in patients with a low serum albumin (Mawer, Miller & Turnburg, 1972).

Although the decrease in protein binding was small and unpredictable it was of pharmacokinetic importance because there was a direct relationship between protein binding and the volume of distribution in normal subjects and those patients with chronic liver disease without ascites (Figure 4). Subjects with a high protein binding had a smaller amount of free drug available for the extensive tissue distribution which this drug has, and hence a smaller volume of distribution. This would result in a greater amount of drug being maintained within the vascular compartment, to be delivered to the liver, allowing a more rapid elimination. As protein binding does not limit the ability of the liver to extract the drug, the plasma protein binding acts as a vehicle for delivering the drug to its site of elimination (Evans & Shand, 1973). Conversely, an increase in free drug from 5% to 30% allows more free drug to be available for distribution into tissues and is associated with a sixfold increase in the volume of distribution. There is a reduction in plasma concentration with a lower rate of delivery of drug to the liver in spite of a similar blood flow and consequently a reduced rate of elimination. The magnitude of these changes would be expected to influence the pharmacodynamic action of drugs which are handled similarly and which are therapeutically active.

The patients with chronic liver disease who had gross ascites had a similar range of protein binding to patients with chronic liver disease without ascites yet had a twofold increase in the volume of distribution for any degree of protein binding. Assuming that the free drug to tissue partition is constant between subjects, and that the plasma volume of distribution of ICG is a measure of plasma protein pool in those patients without ascites, then the plasma protein pool can be calculated in the patients with ascites from the mean data (Figure 4). In patients with chronic

liver disease, the development of ascites was associated with an increase in size of the plasma protein pool; as the degree of protein binding was similar, there was an increased amount of free drug available for tissue distribution and the volume of distribution was higher. Thus the development of ascites has the potential for independently influencing the disposition of a drug.

In conclusion, chronic liver disease tends to influence the three major independent variables which determine the $T_{\frac{1}{2}}$ of (+)-propranolol in such a way that increases in plasma protein pool size, decrease in protein binding and decreases in clearance will all tend to prolong the $T_{\frac{1}{2}}$.

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