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Distribution kinetics and intestinal absorption of practolol in the rat

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Summary

1. After intravenous injection of practolol the blood concentration-time curve is fitted by a bi-exponential function. A two compartment open system model is therefore a minimal requirement in order to describe adequately the distribution of the drug in the body.

2. The parameters of the two compartment model for practolol were determined. The mean values for the 'fast disposition' half life and the 'slow disposition' half life were 0·5 min and 13·3 min, respectively.

3. The rate constants of distribution and elimination were similar in different animals. The volume of the central compartment was related to the weight of the animal.

4. The absorption of practolol from an intestinal site was measured by its appearance in the blood. The rate constant for this process was estimated to be 0·03 min^{-1}.

Introduction

Many drug distribution studies assume that the body has the properties of a single compartment and that a drug mixes freely across all cell membranes so rapidly that the rate of transfer to different regions of the body cannot be measured. Reigelman, Loo & Rowland (1968) examined such a model and discussed some of its limitations. They pointed out that after rapid intravenous injection of a drug, provided that blood samples are taken soon after injection, a plot of its concentration in the blood against time produces at least a bi-exponential curve. The initial part of this curve, the 'fast disposition' phase, has often been ignored, the body being considered as a single compartment. Riegelman *et al.* (1968) therefore proposed that one central and at least one peripheral compartment appeared essential to describe adequately the distribution of a drug in the body. This model was first described by Teorell (1937), and can be represented thus:

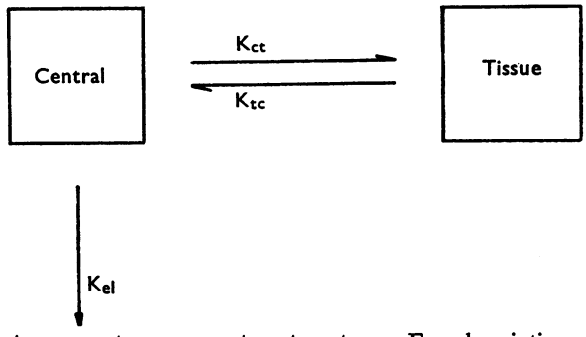


FIG. 1. An open two compartment system. For description see text.

In this model blood is regarded as a component of the central compartment, which may also include some extracellular fluid and the highly perfused tissues such as liver and kidney.

It is assumed that the quantity of drug which is moving out of a compartment by a particular pathway at any instant is proportional to the quantity of drug present in the compartment at that time. Therefore the proportion of drug lost along any pathway per unit of time can be represented by a first order rate constant, k.

Solution of the differential equations from such a model yields the following integrated equation describing the blood concentration-time curve after a single intravenous injection:

$$C_B(t)=Ae^{-\alpha t}+Be^{-\beta t}$$

where $C_B(t)$ is the concentration of drug in the blood at a specified time, t; α and β are both hybrid rate constants. When the concentration is plotted on a logarithmic scale against time, as in Fig. 2, $-\beta/2{\cdot}303$ is given by the slope of the linear part of the curve and B is given by the extrapolated zero time intercept. Resolving the curve into two components by the method of residuals (Riggs, 1963) yields a second linear segment of slope $-\alpha/2{\cdot}303$ and the extrapolated zero time intercept of A.

The distribution of practolol (I.C.I. 50,172) was evaluated in terms of the model in Fig. 1, where k_{ct} and k_{tc} are the first order rate constants of distribution and k_{el} is the sum of the simultaneous processes of metabolism and excretion. The constants A, B, α and β are obtained graphically as described above. These terms are then used with the appropriate equations (see Table 1) to evaluate the parameters of the model.

Once these parameters are known for a particular drug the rate constant, k_a, for absorption into the central compartment from an extravascular site can be determined using the method of Loo & Riegelman (1968). The total amount, $Q(t)$, of drug absorbed at time t is given by:

$$Q(t)=V_cC_c(t)+V_ck_{el}\int_0^t C_c dt+V_TC_T(t)$$

where $C_c(t)$, $C_T(t)$=concentrations in central and tissue compartments at time t (C_c is assumed equal to blood concentrations); V_c, V_T=volumes of central and tissue

TABLE 1. *Equations for calculation of parameters of the open two compartment model of Fig.* 1 (*after Riegelman* et al., 1968)

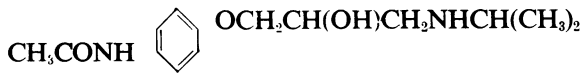
Area under blood concentration-time curve at infinite time:	$A_m=A/\alpha+B/\beta$
Concentration in central compartment (assumed equal to blood concentration) at zero time:	$C_c(o)=A+B$
First order distribution rate constants:	$k_{tc}=\frac{A\beta+B\alpha}{C_c(o)}$
	$k_{ct}=\frac{AB(\beta-\alpha)^2}{C_c(o)^2k_{tc}}$
First order rate constant for elimination:	$k_{el}=C_c(o)/A_m$
Volume of central compartment:	$V_c=\text{Dose}/C_c(o)$
Effective volume of tissue compartment:	$V_T=V_ck_{ct}/k_{tc}$
Volume of distribution at steady state:	$V_{dss}=V_c+V_T$
Area under blood concentration-time curve at time t:	$\text{Area}=\frac{A(1-e^{-\alpha t})}{\alpha}+\frac{B(1-e^{-\beta t})}{\beta}$

compartments (see Table 1); k_{el}=rate constant for elimination, calculated from intravenous data.

The integral is evaluated from the area under the curve of concentration in blood versus time during the experiment. The tissue concentration, $C_T(t)$, is calculated from the blood concentration, using the distribution rate constants, k_{ct} and k_{tc}, determined from experiments in which the drug was given intravenously. The amount absorbed approaches an asymptote as absorption is completed. The rate constant for absorption, k_a, is found from a semi-logarithmic plot of the percentage of the asymptotic value unabsorbed against time.

The object of the present study was (a) to investigate in rats the distribution kinetics of practolol and to ascertain whether or not the kinetics could be defined by the open two-compartment system shown in Fig. 1; (b) to estimate the parameters of the model and to see if they varied markedly between individual animals; (c) it was then hoped to obtain the first order rate constant, k_a, for absorption from the intestine.

Practolol has selective β-adrenoceptor blocking activity and has the following structural formula:



Methods

Male Wistar rats, body weight 350–480 g, were anaesthetized by an intraperitoneal injection of Nembutal (pentobarbitone sodium, 60 mg/kg). The trachea, jugular vein and carotid artery were cannulated; heparin was injected intravenously (125 I.U./100 g body weight); practolol was administered intravenously in 0·9% saline (5 mg/100 g or 10 mg/100 g body weight). The injection was completed within about 10 seconds. Blood samples of about 0·2 ml were collected from the carotid artery at half minute intervals in the first few minutes of the experiment and thereafter at longer intervals.

In experiments concerned with absorption from the intestine the drug was administered directly into the cannulated duodenum in 5 ml phosphate buffer pH 7·4 (10 mg/100 g body weight) or as a suspension in 10 ml normal saline (20 mg/100 g body weight). Blood samples were collected from the carotid artery.

The concentration of practolol in whole blood and aqueous solution was determined as follows. Each blood sample was made alkaline with 0·5 N NaOH and extracted into chloroform; this solvent was then evaporated under reduced pressure. The residue was hydrolysed with 3 N HCl, and the resulting free amine was estimated by the method of Bratton & Marshall (1939). The optical density was read against water using a Hilger-Watts H810 absorptiometer at 585 nm.

Results

After intravenous administration

In all experiments the blood concentration-time curve during the first half-hour period was described by a bi-exponential curve (Fig. 2). The 'fast disposition' half life, $0{\cdot}693/\alpha$ and the 'slow disposition' half life, $0{\cdot}693/\beta$, that is the time required for A and B to decrease to one-half their original value, were 0·5 min and

13·3 min, respectively. The area under the plasma concentration curve at infinite time was proportional to the dose administered for each experiment. These results are listed in Table 2. In some experiments the blood concentration of practolol was found to increase slightly in samples taken more than 20 min after the injection.

The bi-exponential blood curve was interpreted in terms of a two compartment open system. The individual parameters of the model were obtained for each experiment and these results are given in Table 3. The rate of clearance from the

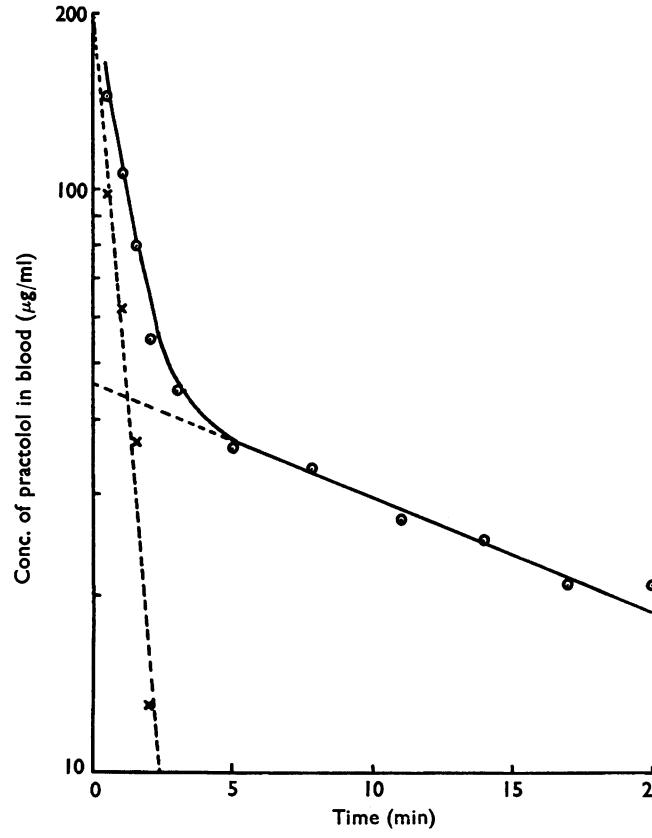


FIG. 2. Blood concentrations of practolol after intravenous injection (5 mg/100 g). Circles, observed results; solid line, calculated curve from $C_B(t)=195e^{-1.555t}+46e^{-0.045t}$; crosses, remaining first term of the function after subtraction of second exponential term.

TABLE 2. *Bi-exponential equation and half lives after intravenous administration*

Dose (mg)	General equation (μg/ml) $(C_B(t)=Ae^{-\alpha t}+Be^{-\beta t})$	Area under plasma curve Dose (min/l.)
34	$C_B(t)=330e^{-1.386t}+66e^{-0.0385t}$	57
40	$C_B(t)=440e^{-1.386t}+122e^{-0.0597t}$	59
38	$C_B(t)=280e^{-1.155t}+116e^{-0.0444t}$	75
34	$C_B(t)=285e^{-1.732t}+148e^{-0.0770t}$	59
20	$C_B(t)=195e^{-1.155t}+46e^{-0.0450t}$	59
16	$C_B(t)=205e^{-1.386t}+54e^{-0.0693t}$	57
Mean half lives (±S.E.)	'Fast disposition' $0.693/\alpha$ (min^{-1})	0·52±0·03
	'Slow disposition' $0.693/\beta$ (min^{-1})	13·27±1·46

central compartment, $k_{el}V_c$, is also listed. The volume of the central compartment was proportional to the weight of the animal (see Table 3).

The fractions of the total dose administered which at any time are either in the central compartment, tissue compartment or eliminated were calculated. These results are shown in Fig. 3.

After intestinal administration

The amount of drug absorbed from the intestine was calculated (Loo & Riegelman, 1968). The asymptote to which the amount absorbed was approaching after 40–50 min was estimated by plotting the amount absorbed against time. The percentage of this asymptote unabsorbed was plotted semilogarithmically against time to derive k_a and it was of the order of 0·03/minute. These results are shown in Table 4 and Fig. 4. When the drug was in phosphate buffer (dose: 10 mg/100 g) the amount detected in the blood was quite low. Those experiments which were carried out with the drug administered as a suspension in saline (dose: 20 mg/100 g) gave higher blood concentrations.

Discussion

During any experiment approximately 2 ml of blood were withdrawn from the central compartment. However, the blood is only part of this compartment, which

TABLE 3. *Individual parameters of an open two compartment model for practolol*

First order distribution rate constant	(i) k_{ct}(min^{-1}): 0·840±0·045
	(ii) k_{te}(min^{-1}): 0·371±0·057
First order elimination rate constant	k_{el}(min^{-1}): 0·212±0·019
Volume of the central compartment	Vc(ml): 79·4±4·86
Volume of distribution at the steady state	V_{dss}(ml): 275·10±30·90
Clearance from the central compartment	$k_{el}Vc$(ml min^{-1}): 16·34±0·626
* $\frac{\text{Volume of central compartment}}{\text{wt of rat}}$	(ml g^{-1}): 0·217±0·015

Results are mean ±S.E. All results are based on 5 d.f.
* This ratio was calculated for each experiment after intravenous injection and used to calculate Vc and V_{dss} for the animals used in the experiments after intestinal absorption.

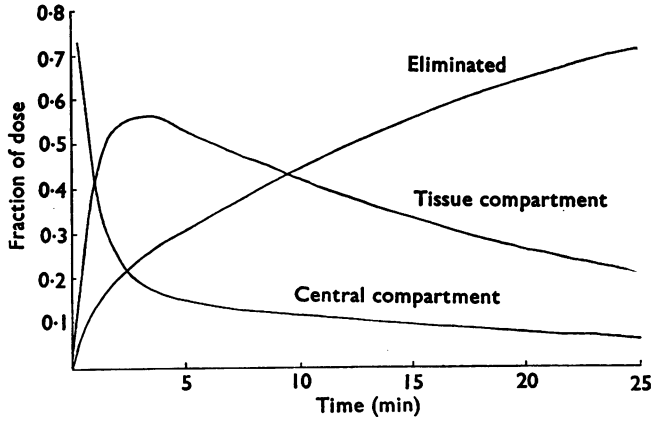


FIG. 3. Distribution of practolol after intravenous injection: the fractions of the total dose administered which at any time are either in the central compartment, tissue compartment or eliminated.

in this case had a volume of 80 ml. Thus the volume remained essentially constant despite the withdrawal of blood.

It has been shown in this laboratory that the blood loss under the conditions described leads to no marked circulatory effects. The blood pressure showed only slight fluctuations as samples were withdrawn, so it was unlikely that circulatory disturbances seriously interfered with the kinetics of redistribution of the drug.

It is evident from the results that if a rigorous sampling schedule is adhered to and blood samples are taken soon after an intravenous injection of practolol then

TABLE 4. *Results after intestinal administration*

Dose (mg)		Calculated V_c (ml)	Time taken to reach 50% absorption, $t\frac{1}{2}$ (min)	$k_a = 0{\cdot}693/t\frac{1}{2}$ (min^{-1})
40	(0·1M phosphate) (buffer, pH 7·4)	88·96	17·0	0·040
80	(suspension in) (0·9% saline)	82·46	20·6	0·034
80	(„) ( )	97·65	28·0	0·025

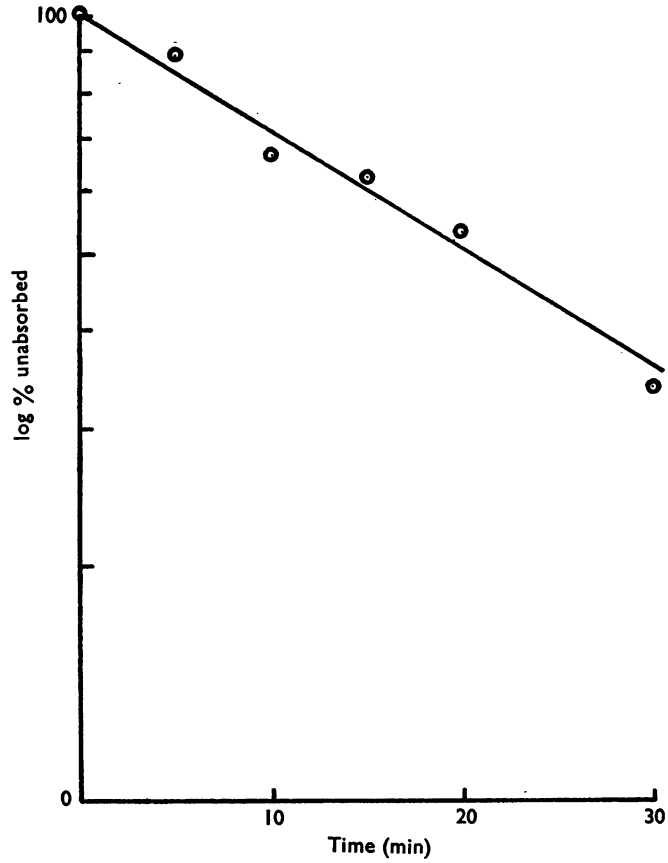


FIG. 4. Log percentage unabsorbed against time for practolol after intestinal administration. Mean results from three experiments on different animals. The half life for absorption is 21 min giving a mean first order rate constant for absorption of 0·033 min^{-1}.

the blood concentration-time curve over the first half hour of an experiment can be described by a bi-exponential function. In some experiments after about 20 min, the concentration of practolol in the blood began to increase, in others it remained steady. The reason for the occurrence of these higher concentrations at this time, which reflect apparently a redistribution of the drug, is not clear. However, it may be due to an enterohepatic circulation, as the drug is known to be excreted in the bile and a similar rise in plasma concentration has been noted in dogs and attributed to recirculation in the bile (D. S. Platt, personal communication). However, if the kinetic analysis is restricted to the first 20 min then the open two compartment model appears adequate to describe the results.

When practolol was given intravenously the rate constants for distribution between the central and peripheral compartments and for the elimination of the drug from the central compartment were very similar for different rats. The area under the plasma concentration-time curve was shown to be directly proportional to the dose administered, which indicates that the rate constants do not change with dose and that over the range studied the kinetics were first order.

k_{ct} was approximately twice the magnitude of k_{tc}. This means that the volume of distribution of the tissue compartment for practolol is double that of the central compartment (Teorell, 1937).

The calculation of k_a, the absorption rate constant from the intestine requires estimates of the volumes of distribution as well as the rate constants of distribution and elimination, which were taken from the results obtained with intravenous administration of practolol. Since there was little variation of the rate constants between animals, the mean value for each rate constant given in Table 3 was used in the calculation of k_a. It was shown in Table 3 that there was a good correlation between the volume of the central compartment and the body weight of each rat, which enabled this volume and the volume of distribution at the steady state to be calculated for those rats used in experiments to determine k_a.

In one experiment the practolol was administered in a solution made up in phosphate buffer (pH 7·4). The resulting analysis of the blood samples yielded very low concentrations of the drug. The aliphatic isopropylamino side chain of the drug has a structure which indicates that it will have a pk_a of about 9, so at pH 7·4 a large proportion of the molecules are in an ionized form. It is generally considered that the intestine is a lipid barrier and that unionized particles dissolve in this lipid phase and move down their concentration gradient by diffusion into the blood. To present a greater number of unionized molecules to this lipid barrier the drug was presented to the intestine as a suspension in saline (pH about 9) and the dose increased to 20 mg/100 g. In this instance, the concentration of practolol in blood was slightly higher than after administration in buffer but was never greater than 30 μg/ml. It is likely that because blood concentrations were low a more accurate estimate of k_a could be obtained from an analysis of the drug excreted in the urine (see Loo & Riegelman, 1968).

To describe the rate of absorption from an intestinal site by a single first order rate constant requires several assumptions. It is necessary to assume (a) that the transfer is irreversible, (b) that when the drug is given as a suspension no further solution from the solid occurs during the period of the experiment, and (c) that the volume of the solution remains constant (that is, no water is absorbed). The semi-logarithmic plot shown in Fig. 4 indicates that a single first order rate process

described the absorption of practolol, so that these assumptions appear to be justified.

Finally, it should be noted that the assay method would detect the deacetylated metabolite of practolol. However, blood samples taken 30 min after intravenous injection of the drug and assayed with the omission of the hydrolysis step gave no evidence of this metabolite. It is concluded that in the short experiments reported it is most unlikely that substances other than practolol were assayed.

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