

THE DISPOSITION OF DIPHENHYDRAMINE AND FOUR OF ITS ANALOGUES IN RABBITS AND IMPLICATION FOR STRUCTURE-ACTIVITY RELATIONSHIP STUDIES IN DRUG DESIGN

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ABSTRACT

The disposition of diphenhydramine (I) and four of its ring substituted analogues, 4-bromodiphenhydramine (II), 4-methyldiphenhydramine (III), 2-methyldiphenhydramine (IV), and 4-*t*-butyldiphenhydramine (V), was investigated in the rabbit, during and after intravenous infusion.

The concentration of each analogue was determined by gas-liquid chromatography (GLC) and the disposition parameters of clearance, volume of distribution, and elimination rate constant determined. These parameters were found to vary within the series, clearance increased in the order $I < IV < III < II < V$ and volume of distribution in the order $III < I < IV < V < II$. These changes correlated with the Hansch hydrophobic substituent parameter: for clearance $r = 0.97$, for volume $r = 0.7$.

The implication of these changes for the design of studies investigating the effects of structure on pharmacological response are discussed.

KEY WORDS Drug disposition Diphenylethanolamine Structure-Activity relationships
Diphenhydramine

INTRODUCTION

In recent years there have been many attempts to correlate the relative activities of drugs with their molecular structure. Progress in this area has led to the routine use of structure activity relationship (S.A.R.) studies in the development of new drugs. Generally, these studies are carried out by relating the pharmacological activity of members of a series of compounds, observed at some fixed time after a standard dose, to their physico-chemical properties. The results of such studies are assumed to reflect the effects of chemical structure on the interaction of the compounds with the bio-receptor.

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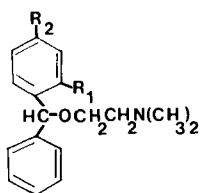
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Implicit in the application of the S.A.R. results to drug design is the assumption that whilst small changes in structure could lead to potentially large changes in intrinsic activity or affinity within a group of similar compounds, they would produce only comparatively minor changes in their disposition. It would follow that equimolar doses of a series of structurally-related analogues should lead to similar concentrations at the biologically active site. If, however, changes in structure were to affect disposition to a significant extent then, as pointed out by Notari,¹ in the interpretation of S.A.R., it would be important to consider the relative concentrations/time profiles of the different compounds as well as their relative activity in the biophase. This concentration, however, cannot be readily determined for drugs which act reversibly, the plasma concentration/time profile is related to the concentration/time profile in other tissues and could be used for this purpose. If both disposition and intrinsic activity or affinity are affected by changes in structure, then the onset, duration and intensity of effect for any member of the series will depend not only on the interaction of the drug with the biological receptor but also on its availability to the biologically active site.

There have been few attempts to test the validity of these assumptions by studying the disposition of a series of structurally-related compounds.¹⁻³ Those studies which have been reported have shown marked differences in disposition within such a series. For example, in an investigation of the disposition of three sulphonamides, sulphasomidine, sulphamethazine, and sulphathiazole, in the rabbit² a fourteen-fold difference in clearance and two-fold difference in apparent distribution volume were reported. Similarly, marked differences in the disposition of a series of substituted benzoylformic and mandelic acids have been reported by Amin and Nagwekar.³ The disposition of several barbiturates has been investigated in relation to their structure.⁴⁻⁶ It was found that clearance and apparent volume of distribution of the members of the series were linearly related to the partition coefficient. Previous reports, therefore, suggest that small changes in structure can have a marked effect on drug disposition. This hypothesis was investigated using the series of diphenylethanolamine antihistamines shown in Figure 1. The disposition of these compounds was studied in rabbits during and after intravenous infusion.



$R_1 = R_2 = H$	Diphenhydramine (I)
$R_1 = H, R_2 = Br$	4-Bromodiphenhydramine (II)
$R_1 = H, R_2 = CH_3$	4-methyldiphenhydramine (III)
$R_1 = CH_3, R_2 = H$	2-methyldiphenhydramine (IV)
$R_1 = (CH_3)_3C, R_2 = H$	2- <i>t</i> -butyldiphenhydramine (V)

Figure 1. Structures of the five diphenylethanolamine antihistamines investigated

MATERIALS AND METHODS

Materials

Diphenhydramine hydrochloride (I), 4-bromodiphenhydramine hydrochloride (II), and 4-methyldiphenhydramine hydrochloride (III) were kindly donated by Parke Davis and Co. Ltd., Pontypool, U.K.. 2-methyldiphenhydramine hydrochloride (IV) was supplied by Arthur H. Cox and Co. Ltd., Rushington, West Sussex, U.K., and 2-butyldiphenhydramine (V) was a gift from Dr. Harms of Brocades N.V., Holland.

Disposition studies

Male New Zealand rabbits weighing between 2.5 and 4 kg were used. Mild anaesthesia was induced and maintained by intramuscular injection of Etomidate (Janssen Pharmaceuticals). A paediatric butterfly infusion set (27G \times 1 cm needle) attached to a Sage Instruments Model 355 syringe pump was primed with drug solution, the needle was inserted in a marginal ear vein, and the drug infused at a rate of about 2 mg h^{-1} over 4–6 h using 2 mg ml^{-1} solution. The infusion flow rate was accurately measured by weighing timed collections from an identical infusion assembly operating from the same pump. An in-dwelling catheter was inserted into a marginal ear vein of the other ear and blood samples (2 ml) were withdrawn at intervals during and after the infusion. An aliquot of the solution used for the infusion was assayed for antihistamine content. The disposition of each compound was investigated in three out of a group of six rabbits such that each rabbit received a least three compounds but none received all five compounds. A recovery period of 1 month was allowed between infusions.

Analysis of blood samples

The blood samples were collected in heparinized tubes and plasma obtained after centrifugation of the blood samples for 15 min. The antihistamine concentration in the plasma samples was determined by GLC using a Perkin Elmer F17 model chromatograph equipped with an alkali flame ionization detector. The stationary phase used for the analyses was 3 per cent OV17 on Chromosorb W (80–100) (Phase Separations Ltd., Queensferry, U.K.). The plasma samples were spiked with the appropriate internal standard given in Table 1, made alkaline with 5N NaOH (0.1 ml) and extracted successively with two volumes of *n*-heptane (12–15 ml). The organic phase was separated and evaporated to dryness with heating under a stream of nitrogen. The inside of the tube was washed with acetone (0.2 ml) which was then removed by evaporation. The residue was re-dissolved in acetone (30 μ l) and an aliquot (5 μ l) injected into the GLC column. Assay details are summarized in Table 1. Antihistamine concentrations were determined from a suitable calibration curve except for the assay of (V). In this case, because of day-to-day variations in detector response to (V) standards were run simultaneously. The variance of each assay was

Table 1. Summary of the analytical methods used for determination of the diphenylethanolamine antihistamines in plasma

Compound	Column temperature (°C)	Injector temperature (°C)	Internal standard	Drug retention time (min)	Internal standard retention time (min)	Concentration of measurement (ng ml ⁻¹)	Reproducibility Coefficient variation (%)	Minimum assayable amount (nmol)
I	215	250	IV	3.4	4.0	103 35	1.2 6.0	0.05
II	240	275	IV	4.0	3.2	98 28	2.1 5.2	0.099
III	225	250	I	2.5	1.8	102 31	1.8 11.9	0.119
IV	215	250	I	4.0	3.4	97 31	2.7 4.7	0.082
V	265	300	I	1.7	1.0	133 42	7.7 9.4	0.045

assessed at two concentrations by spiking five aliquots of plasma (2 ml) with the appropriate compound and proceeding as described above. The minimum assayable quantity that is the amount giving a signal three times that of the background, was also assessed for each compound.

The blood/plasma partition ratio (λ) was determined by spiking three blood samples (2 ml) with a known amount of antihistamine in plasma. The fraction excreted unchanged for each compound was determined by collection of the urine for 3 days after a 10 mg intravenous bolus dose. The urine was assayed for the antihistamine using the method previously described for blood samples but omitting the centrifugation of the blood sample.

DATA ANALYSIS

Plasma concentrations were standardized to an infusion rate of 7834 nmol h^{-1} (equivalent to an infusion rate of 2 mg of (I) base per hour) and a body weight of 3 kg. The pharmacokinetic model which most appropriately described the data was selected after fitting integrated equations derived from both one- and two-compartment open model systems with elimination occurring from the central compartment to the plasma concentration data. The programme NONLIN⁷ was used to fit the equations (1), (2), and (3)⁸ to the unweighted data. Using this program data points obtained during the infusion and post-infusion could be fitted simultaneously using equations (1) and (2) for a one-compartment model and equation (3) for a two-compartment model. The selection of a model to describe the data for each compound was based on:

- (a) The scatter of the observed data points about the model-predicted curve.
- (b) Comparison of the sum of squared deviations of the fitted equation, and
- (c) Visual inspection of the residual points.

A more detailed description of these criteria is given by Boxenbaum *et al.*⁹ After a selection of the most appropriate model, disposition parameters for each compound were calculated. The unweighted data were used for model selection.

$$C_p = \frac{R_0}{kV}(1 - e^{-kt}) \quad (1)$$

$$C_p = \frac{R_0}{kV}(1 - e^{-kt})e^{-k(t-T)} \quad (2)$$

$$C_p = \frac{R_0(k_{21} - \alpha)(1 - e^{\alpha T})e^{-\alpha t}}{V_c\alpha(\alpha - \beta)} + \frac{R_0(\beta - k_{21})(1 - e^{\beta T})e^{-\beta t}}{V_c\beta(\alpha - \beta)} \quad (3)$$

where

C_p = Plasma concentration (nmol l^{-1})

R_0 = rate of infusion (nmol h^{-1})

- k = elimination rate constant (h^{-1})
 V = volume of distribution (l)
 k_{21} = intercompartmental transfer rate constant (h^{-1})
 α = first disposition rate constant (h^{-1}) rapid phase
 β = second disposition rate constant (h^{-1}) terminal phase
 V_c = volume of central compartment (l)
 t = time from start of infusion (h)
 T = duration of infusion (h)

During infusion $T = t$ and varies with time, when the infusion ceases T becomes a constant corresponding to duration of infusion.

RESULTS

The conditions used, the coefficients of variation, and minimum quantifiable amounts for each assay are summarized in Table 1. Examples of plasma concentration profiles for each compound are shown in Figure 2.

The disposition parameters for each compound were obtained by fitting the equations (1), (2), and (3) to the data for each rabbit and to the combined data for each compound obtained from these rabbits. Values for the disposition parameters in individual rabbits and for the combined data from three rabbits who received (III) are given in Table 2, there is no significant difference between the values obtained by the two methods ($p < 0.01$).

Based on the criteria outlined under 'Data Analysis', the simplest model was selected for each compound. A one-compartment model adequately described the data for (I), (III), (IV), and (V). However, a two-compartment model was judged to give a better description of the data for (II). As an example, the observed and model-predicted plasma profiles for (II) are shown in Figure 3.

Drug disposition can be quantitatively compared using the pharmacokinetic parameters of plasma clearance (Cl), half-life ($t_{1/2}$), elimination rate constant (k), fraction of dose excreted unchanged (fe), blood clearance (Cl_b), and volume of distribution. These were calculated from the equation which best described the data using the methods of Gibaldi and Perrier.⁸ The steady-state volume of distribution (V_{dss}) was used for (II) since, unlike other estimates of volume of distribution it is independent of the elimination process and, therefore, reflects only differences in distribution. V_{dss} can be calculated from the equation best describing the data using the methods of Gibaldi and Perrier.⁸

The fraction of dose excreted unchanged was estimated from the amount of unchanged drug excreted in 3 days, equivalent to at least twenty plasma half-lives, in which time, all unchanged drugs would have been eliminated.

Plasma clearance may be influenced by differences in the whole blood/plasma concentration ratio (λ) therefore blood clearance (Cl_b) was also calculated. These pharmacokinetic parameters are given in Table 3.

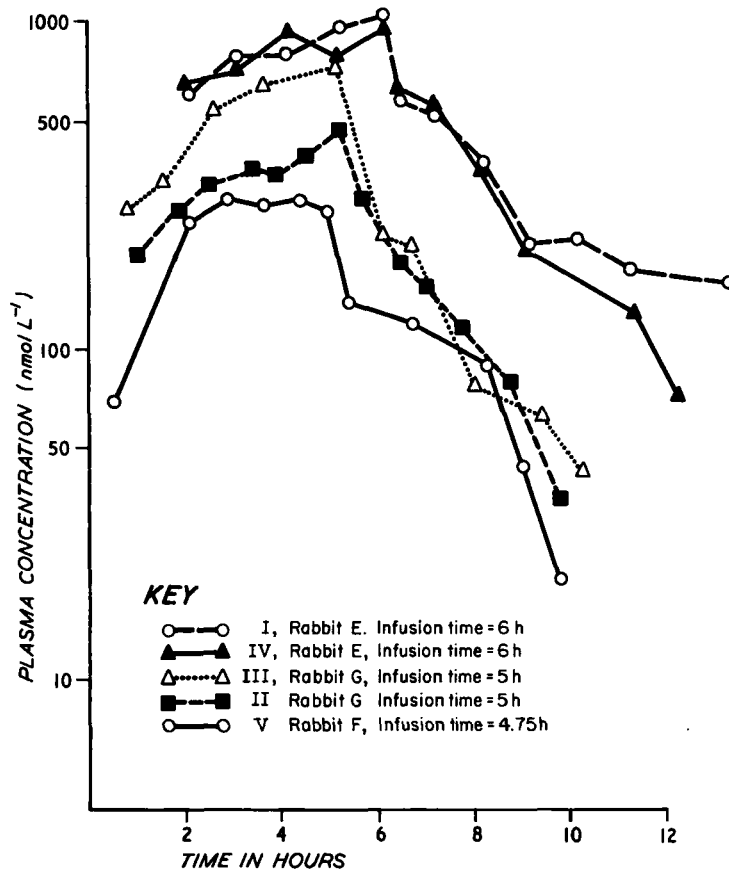


Figure 2. Examples of plasma concentration profiles for five diphenylethanolamine antihistamines during and after infusion in rabbits

Table 2. Computer estimates of V and k which give best fit to the individual data and combined data obtained after infusion of III

	Rabbit H*	Individual data Rabbit G	Rabbit F	Combined data
V (l)	15.7 (1.95)	10.6 (1.52)	9.3 (0.84)	11.4 (0.97)
k (h^{-1})	0.71 (0.09)	1.11 (0.16)	1.0 (0.09)	0.94 (0.08)

* Numbers in parentheses are standard deviation.

DISCUSSION

The results given in Table 3 indicate that substitution of one of the phenyl rings of (I) results in a group of compounds with differences in their concentration

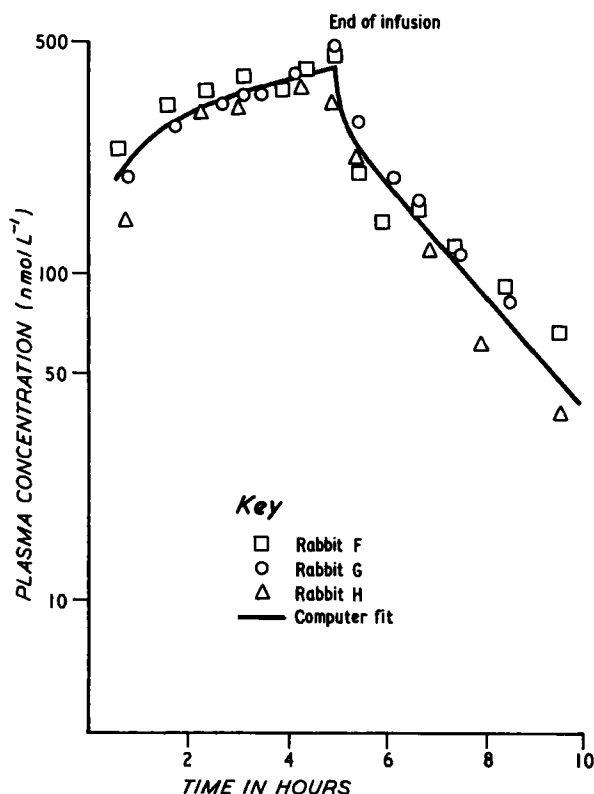


Figure 3. Observed plasma concentrations of (II) during and after constant intravenous infusion in three rabbits. Data standardized to an infusion rate of $2614 \text{ nmol h}^{-1} \text{ kg}^{-1}$

time profiles. These are given for each compound, after a $2614 \text{ nmol h}^{-1} \text{ kg}^{-1}$ infusion administered for 5 h, in Figure 4.

Substitution affects both clearance and volume of distribution. Clearance is increased for all compounds. Since renal elimination is a minor pathway of excretion the calculated plasma clearance is similar to the non-renal plasma clearance of each compound. The plasma clearance can be converted to blood clearance using the plasma/red cell partition ratio (λ). The blood clearance (Cl_b) of (II) and (V) are in excess of the hepatic blood flow of a 3 kg rabbit which is $70\text{--}170 \text{ ml s}^{-1}$. This implies that extra hepatic sites of metabolism may be of importance in the elimination of these compounds.

There is an excellent correlation between blood clearance and the Hansch hydrophobic parameter π ,¹⁰ a measure of lipophilicity. This relationship is given in equation (4).

$$Cl_b = 117\pi + 90 \quad (r = 0.97, R = 5) \quad (4)$$

Table 3. Disposition parameters for the ethanalamine antihistamines investigated in the rabbit

Compounds	Disposition parameters*						
	V_{ss} (l)	k (h^{-1})	$t_{1/2}$ (h)	Cl ($ml\ min^{-1}$)	Cl _b ($ml\ min^{-1}$)	λ	f_{∞}
I	16.5 (1.57)	0.44 (0.05)	1.57	121	88	1.38	0.0302
II	31.8†	0.40 (0.08)‡	1.73	280	219	1.28	0.0086
III	11.5 (0.98)	0.94 (0.08)	0.74	180	140	1.29	0.0066
IV	22.0 (2.04)	0.42 (0.04)	1.65	154	143	1.08	0.0074
V	30.1 (3.80)	0.78 (0.11)	0.89	391	277	1.41	0.0069

* Numbers in parentheses are standard deviations, see text for definition of abbreviations used.

† Hansch's hydrophobic substituent parameter.

‡ Standard deviation not available for this derived parameter.

§ The value given is that of β , the slope of the terminal phase.

††

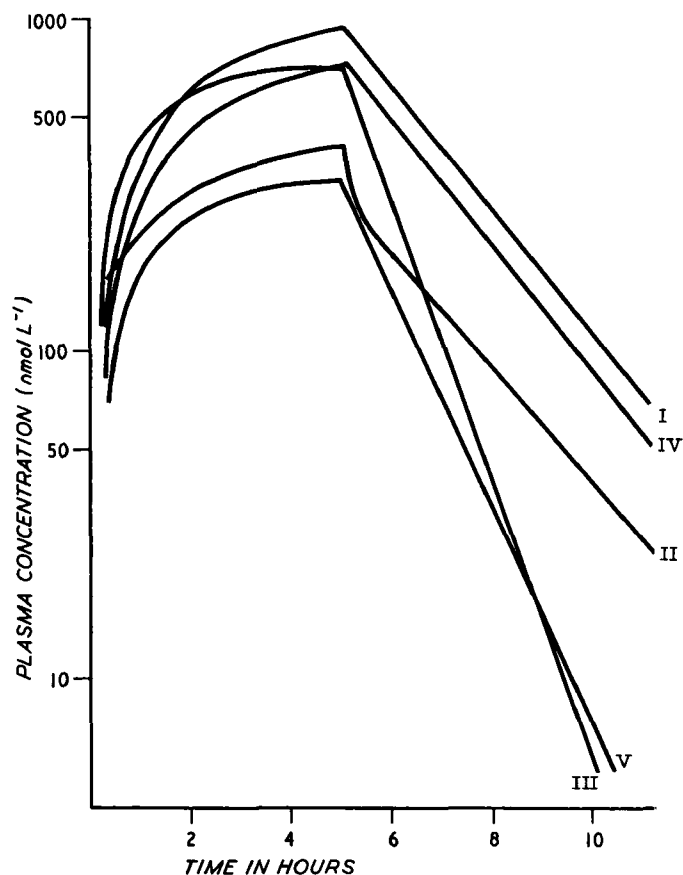


Figure 4. Computer-predicted plasma concentrations for five diphenylethanolamine antihistamines in a rabbit during and after intravenous infusion at a rate of $2614 \text{ nmol h}^{-1} \text{ kg}^{-1}$

This relationship suggests that increased lipophilicity favours transfer to the site of elimination and enhanced rate of metabolism.

The volume of distribution varies two-fold within the series of compounds studied. The variation in protein binding in the series was not determined and this may be an important determinant of distribution. However, all the compounds have large apparent volumes of distribution suggesting extensive tissue uptake. There is a correlation between volume of distribution V_{dss} and π which is given in equation (5).

$$V_{\text{dss}} = 9.9 + 15.3 \quad (r = 0.7, n = 5) \quad (5)$$

From these results it can be seen that substitution at a single position of one of the phenyl rings of (I) leads to compounds which have different pharmacokinetic properties to (I). Substitution produces changes in both clearance and

volume of distribution. Therefore, estimation of half-life alone could be a misleading indicator of the extent of changes in disposition parameters and the resulting changes in the plasma concentration/time profile. This effect would be seen in the concentration/time profile after a bolus injection of each compound to a 3 kg rabbit. This profile would be similar to the post-infusion data shown in Figure 4. Estimation of the relative pharmacological activity of these compounds could, therefore, be affected by the timing of the experiment unless these were carried out under steady-state conditions with regard to the analogues under investigation. These results would suggest that in drug design experiments it would be relevant to relate the magnitude and time course of activity to the drug concentration in plasma at the time of testing. In this way high activity due to favourable disposition characteristics could be separated from that due to increased affinity or activity at the receptor site.

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