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PHARMACOKINETIC STUDIES WITH ATENOLOL IN THE DOG

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ABSTRACT

The pharmacokinetics of the cardioselective β-adrenoreceptor blocking agent atenolol have been determined following intravenous and oral dosing to the dog. After intravenous administration at 200 mg the blood levels of parent drug were found to decay tri-exponentially with a final elimination phase half-life of about 4·5 h. The volume of distribution for the central compartment was 40 per cent body weight and the whole body volume of distribution was 160 per cent body weight. The percentage urinary recovery of parent drug was 83 per cent.

Following oral dosing at 400 mg (as a solution and as a clinical trial tablet) the percentage urinary recovery was 65 per cent and the half-life extended slightly to between 5 and 6 h. The peak blood levels were however very similar for the two formulations (17 and 15 μg/ml for the solution and tablet respectively) and occurred at the same time (1–2 h after dosing). The total areas under the blood concentration time curves were similar and the values (100 and 104 $\mu g ml^{-1} h$ respectively) agreed well with that anticipated on the basis of the intravenous data. It was concluded that the two formulations were bioequivalent and that following oral dosing atenolol was almost completely absorbed with little metabolism or biliary excretion.

Following chronic oral dosing at 50, 100, and 200 mg/kg/day the systemic blood levels were found to increase with dose at all time points throughout the study. There was no sex or dose dependency of the half-life and its value on chronic dosing was very similar to that on acute dosing. The dose dependency of the area under the blood concentration time curves was reflected in the plateau blood levels and there was very good agreement between the experimental values and the theoretical relationship based on the acute pharmacokinetic data. In accordance with the half-life there was no accumulation at any of the dose levels studied. Thus it can be concluded that atenolol obeys linear pharmacokinetics over the dose range studied.

KEY WORDS Pharmacokinetics Dog Acute and chronic Intravenous and oral

INTRODUCTION

Atenolol (Tenormin®)† is a β-adrenoreceptor blocking agent developed by the Pharmaceuticals Division of Imperial Chemical Industries PLC. In animal

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studies atenolol has been shown to be cardioselective and devoid of intrinsic sympathomimetic and membrane stabilizing activity.[1-3]

The purpose of the study was to determine the blood level profile of atenolol following intravenous and oral administration to the dog and to monitor the degree of absorption in a long term dog toxicity test (TFD/140).

MATERIALS AND METHODS

Drug formulations

Atenolol was used in the preparation of the following formulations

Formulation A—a 40 mg/ml solution of atenolol in physiological saline with citric acid added to aid solubilization. The volume administered to each dog was 5 ml equivalent to a dose of 200 mg per dog.

Formulation B—a hard gelatin capsule containing 8 ml of a 5 per cent w/v solution of atenolol in water buffered with an equal weight of citric acid and equivalent to 400 mg of drug.

Formulation C—a hard gelatin capsule containing 4 × 100 mg clinical trial atenolol tablets.

Formulation D—a 200 mg toxicity tablet for oral administration to the dog. A number of tablets were contained in a hard gelatin capsule such that the dose administered to the dogs was either 50 mg/kg/day (Group II), 100 mg/kg/day (Group III) or 200 mg/kg/day (Group IV).

Study design

Intravenous study. Various male beagle dogs weighing between 12 and 19 kg were used in this work. In particular for the intravenous study six male beagles (code numbered 119, 355, 356, 623 (twice), 627, and 969) received formulation A above following overnight fast. Whole blood samples were collected into oxalated blood tubes at 5, 10, 15, 20, 30, and 45 min, 1, 1·5, 2, 3, 4, 5, 7, 12, 24, 30, and 48 h post-dosing. Urine samples were collected over the time intervals 0–24, 24–48, and 48–72 h post-dosing together with the cage wash at termination.

Comparative bioavailability study. Three of the male beagles used for the intravenous study (355, 356, and 627) plus one other (code numbered 305) were each dosed in a two by two crossover with formulations B and C above. Whole blood samples were collected into oxalated blood tubes at 30 min and 1, 1·5, 2, 3, 4, 5, 6, 7, 24, 30, and 48 h post-dosing. The total urinary recovery over the 72 h post-dosing period, together with cage wash, was also collected.

Toxicity study. During the 12 month chronic oral toxicity study with atenolol (TFD/140) three groups of dogs comprising five males and five females per group were dosed with formulation D above. Whole blood was collected into oxalated tubes predose, and at 1, 3, 5, 7 and 24 h post-dosing at 1 month, 3 months, 6 months, 9 months, and 12 months into the study.

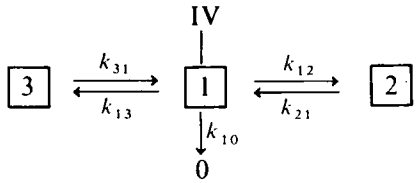
In each study all the samples were stored at −20° prior to analysis.

Sample analysis. Most of the blood and urine samples were analysed using a fluorescence technique, but some were analysed using gas liquid chromatography.[4] An outline of the fluorescence method follows:

One millilitre of oxalated whole blood or urine was mixed with 2 ml of 0·1 N NaOH solution and 8 ml of a 50 per cent v/v cyclohexane/*n*-butanol solvent mixture. After being mechanically shaken for 15 min and centrifuged at 1500 rpm for 5 min to clarify the phases, 7·5 ml of the organic phase was transferred to a clean tube containing 2 ml of 0·1 N HCl. If the emulsion in the organic phase was unbroken on centrifugation, the tube was plunged into a Drikold/Acetone freezing mixture and then re-centrifuged. The stoppered tube was again mechanically shaken for 15 min, centrifuged for 5 min and then the organic layer aspirated and discarded.

The optical density of the aqueous phase was determined on an MPF-3 fluorescence spectrophotometer using an activation wavelength of 235 nm and scanned from 280 to 320 nm to measure the emission wavelength at 300 nm. The limit of detection was between 0·5 and 1·0 μg/ml.

Theoretical. All pharmacokinetic methods for the estimation of bioavailability are both model—and assumption—dependent. For atenolol in the dog a three-compartment model was used and it can be represented by the following:[5]



where the rate constants (k) govern transfer of drug and/or metabolites from one compartment to another. For an intravenous injection the drug is given as a bolus into the central compartment (1) from which it transfers to either of the two tissue compartments (2 and 3); the drug being eliminated from the central compartment. Solution of the differential equations governing the system gives the concentration of drug in the blood at time t, $c\ (t)$.

$$c(t) = P.e^{-\Pi t} + A.e^{-\alpha t} + B.e^{-\beta t}$$

where

$$P = \frac{c_p^0(k_{21} - \Pi)(k_{31} - \Pi)}{(\Pi - \beta)(\Pi - \alpha)}$$

$$A = \frac{c_p^0(k_{21} - \alpha)(k_{31} - \alpha)}{(\Pi - \alpha)(\beta - \alpha)}$$

and

$$B = \frac{c_p^0(k_{21} - \beta)\,(k_{31} - \beta)}{(\Pi - \beta)\,(\alpha - \beta)}$$

and $c(t)$ is the concentration of drug in the blood at time t; c_p^0 the concentration in the blood immediately after the bolus dose has been administered and α, β and Π are all functions of the individual rate constants. Thus, by measuring the blood levels following an intravenous injection and plotting them on a semi-logarithmic scale, the parameters Π, α, β, c_p^0, A, B, and P can be calculated and used to evaluate the individual rate constants.

RESULTS

Intravenous study

The mean atenolol blood levels ($\pm$S.E.) obtained after the 200 mg intravenous dose to the dogs is illustrated in Figure 1 and the feathering appropriate to this curve is illustrated in Figure 2 for the time period 0–6 h post-dosing. The blood level decay was shown to follow a tri-exponential relationship of the form.

$$c(t) = P.e^{-\Pi t} + A.e^{-\alpha t} + B.e.^{-\beta t}$$

where

$$P = 19{\cdot}07\,\mu g/ml;\ \Pi = 6{\cdot}356\,h^{-1}$$
$$A = 7{\cdot}18\,\mu g/ml;\ \alpha = 1{\cdot}149\,h^{-1}$$
$$B = 6{\cdot}48\,\mu g/ml;\ \beta = 0{\cdot}158\,h^{-1}$$

Thus, at this particular dose level, the initial distribution phase has a half-life of 0·11 h; the secondary distribution phase a half-life of 0·60 h; and the final elimination is associated with a half-life of 4·39 h. The area under the blood level curve to infinity, determined theoretically, was 50·3 $\mu g ml^{-1} h$. We attempted to fit a three-compartment model for individual dogs using weighted non-linear least squares. As a consequence some dogs were fitted but for others the algorithm did not converge. For those dogs which did converge the three compartment system showed a better fit than a two compartment system.

From this study the volume of the central compartment V_P, defined as:

$$V_P = \frac{\text{dose}}{c_p^0}$$

where c_p^0 is the concentration in the central compartment at zero time, i.e. the y-axis intercept in Figures 1 and 2 for the line $c(t) = Pe^{-\Pi t} + Ae^{-\alpha t} + Be^{-\beta t}$ was found to be 6·111 or 40 per cent body weight. The whole body volume of distribution, defined as

$$V_\beta = \frac{\text{dose}}{\beta.\ \text{AUC}(\infty)}$$

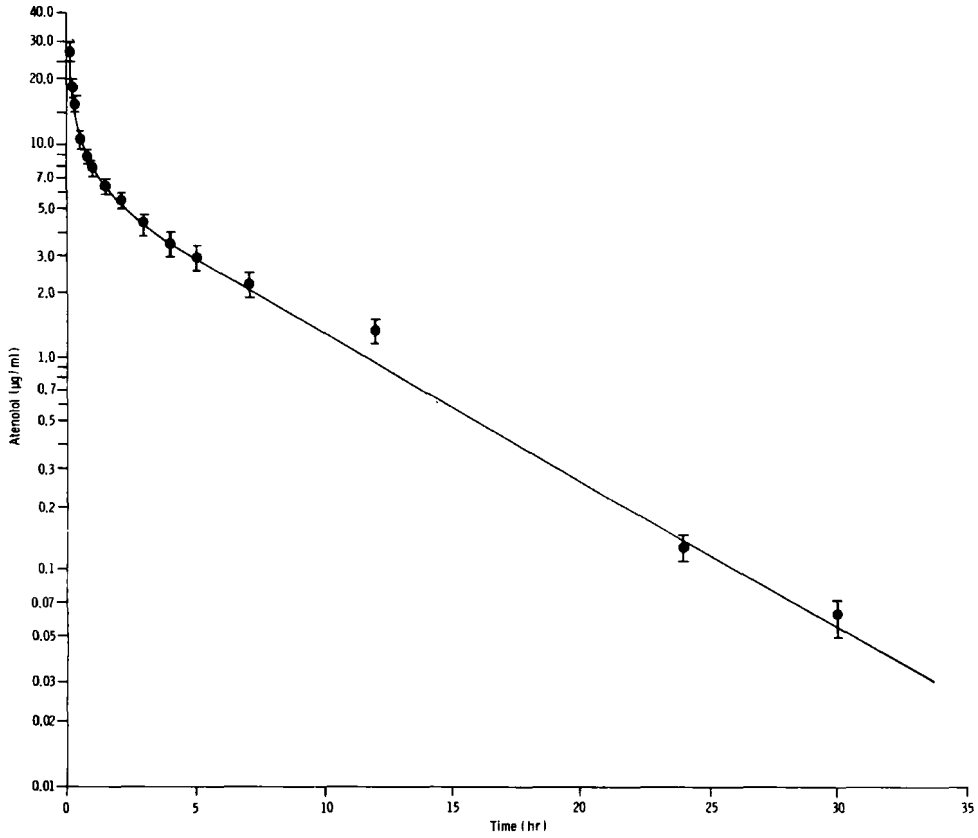


Figure 1. Levels of atenolol (μg/ml) ± s.e. in whole blood of dogs dosed 200 mg intravenously

where β is the slope for final phase elimination was found to be 25·171 or 160 per cent whole body weight.

The values for Π, α, β, c_p^0, A, B, and P were used to calculate the various rate constants as outlined in the theoretical section and the results obtained, using a basic computer program, are as follows:

$$k_{12} = 0{\cdot}954\,h^{-1}$$
$$k_{21} = 0{\cdot}571\,h^{-1}$$
$$k_{13} = 2{\cdot}381\,h^{-1}$$
$$k_{31} = 3{\cdot}106\,h^{-1}$$
$$k_{10} = 0{\cdot}651\,h^{-1}$$

The mean recovery of atenolol in the urine (Table 1) was 166 ± 6 mg (S.E.) or 83 ± 3 per cent of the original dose given. This result suggests that either the drug is only slightly metabolized in the dog, or that a small degree of excretion

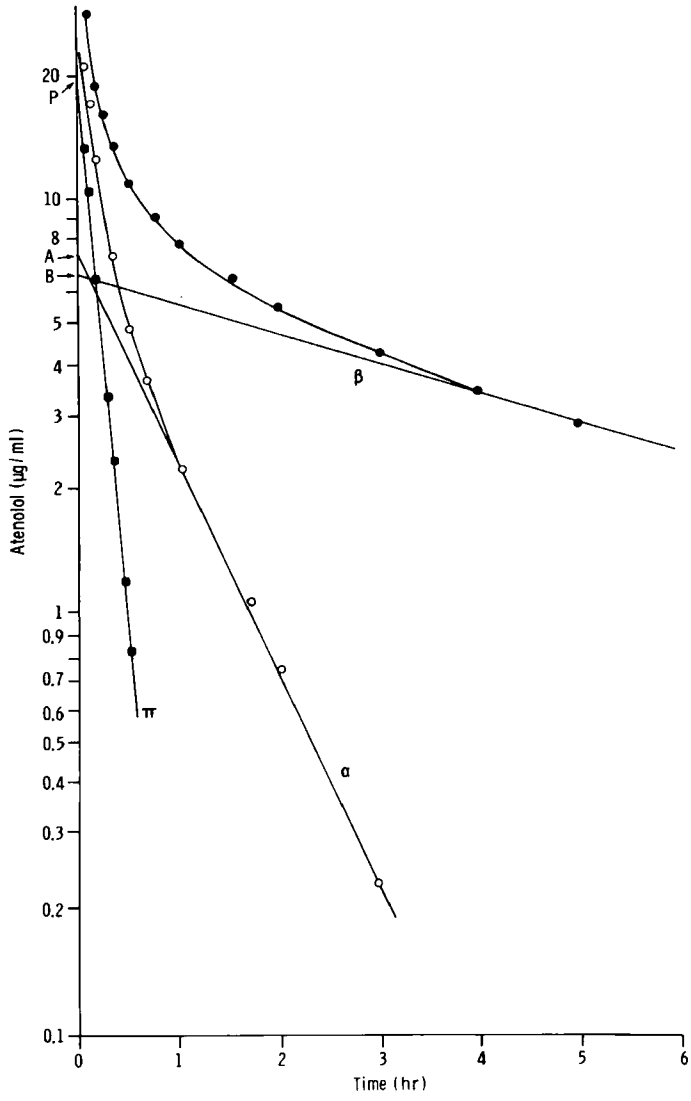


Figure 2. Average levels of atenolol (µg/ml) in whole blood of dogs dosed 200 mg intravenously (0→6 h time points only)

into the faeces via the bile is taking place. Most of the excretion (75 per cent of the dose) takes place within the first 24 h.

Comparative bioavailability study

The mean atenolol blood levels obtained after the administration of formulations B and C are illustrated in Figure 3 and the mean urinary recoveries in Table 2. Peak blood levels of 17 and 15 µg/ml for the solution and tablet occur

Table 1. Mean recovery of atenolol (mg) in urine of dogs following a 200 mg intravenous dose

Parameter	Time interval (h) 0–24	24–48	48–72	Total (%)
Mean	152	11	2·6	83
S.D.	14	9	2·0	8
S.E.	5	3	0·8	3

S.D. = Standard deviation.
S.E. = Standard error.

at 1 and 2 h post-dosing respectively i.e. the atenolol appears to be absorbed slightly faster from the aqueous solution than from the clinical trial tablets; the peak shift being about 1 h on average. The distribution phase lasts for about 4 h for both formulations, and the final elimination phase has a half-life of 4·9 and 5·8 h on average for the solution and tablet respectively. The areas under the average blood level curves were measured by the trapezoidal rule up to 6 h and the remainder calculated using the expression $c(6)/\beta$. The total values obtained were 100·0 and 104·2 μg ml^{-1} h for the solution and tablet respectively. When one takes into account that on intravenous administration 200 mg absorbed was equivalent to 50·3 μg ml^{-1} h one can calculate that 369 mg and 384 mg of drug were absorbed from the solution and tablet respectively. Thus, although there is a slight difference in peak time for the two blood level curves the total amount of drug absorbed is the same for both formulations and furthermore, within experimental error, equal to the dose administered.

The average recovery of unchanged drug over the excretion period (72 h) was 258 mg and 256 mg i.e. 65 per cent of the original dose for both the solution and tablet. This confirms that the total amount of drug absorbed is the same for both formulations. The urine recoveries of unchanged drug are slightly lower than those obtained following intravenous dosing.

Table 2. Mean recovery of atenolol (mg) in urine of dogs following 400 mg oral doses

Formulation	Parameter	Time interval (h) 0–72	Total (%)
B	Mean	258	65
	S.D.	34	9
	S.E.	17	4
C	Mean	256	65
	S.D.	21	5
	S.E.	11	3

S.D. = Standard deviation.
S.E. = Standard error.

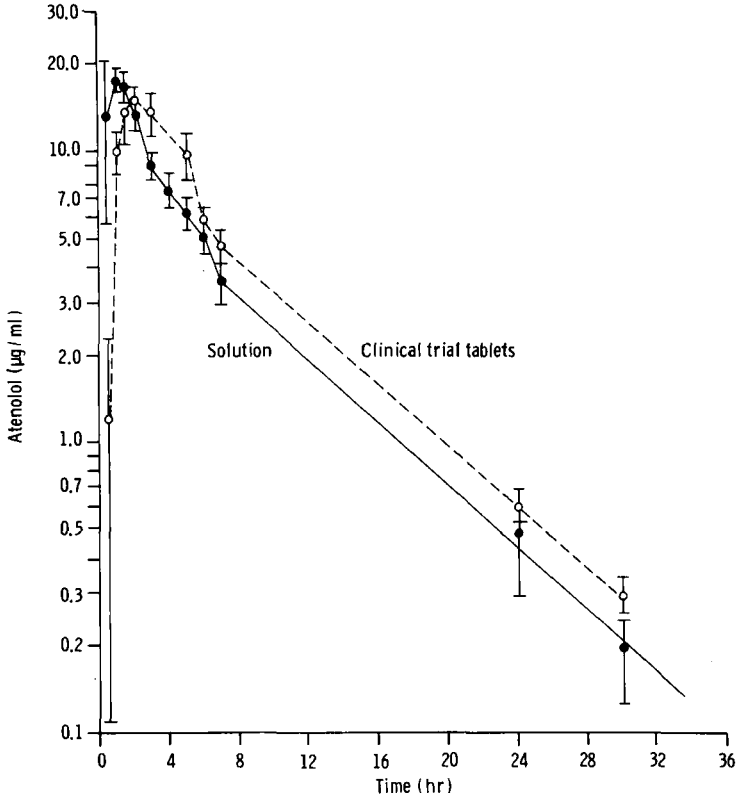


Figure 3. Average levels of atenolol (µg/ml) ± s.e. in whole blood of 4 dogs dosed 400 mg orally as (a) solution and (b) clinical trial tablets

Safety evaluation

The mean and standard error atenolol levels for the combined male and female dogs are illustrated in Figure 4 for sampling at 1, 3, 6, 9, and 12 months into the study.

The individual values for the elimination phase half-lives were compared by '*t*'-test and it was found that with three exceptions out of the twenty tests made there was no significant difference in the half-lives between the male and female dogs i.e. one can conclude that there was no sex dependency of the kinetics. Furthermore with three exceptions out of twenty (not the same three as above) there was no significant difference in the half-lives between doses i.e. one can conclude that there was no dose-dependency of the kinetics. The average half-life at each of the sampling points together with its standard error was therefore calculated and the results are shown in Figure 5. Clearly during the trial the elimination phase half-life is virtually constant between 5 and 7 h which is consistent with the data obtained following the single oral dose experiments.

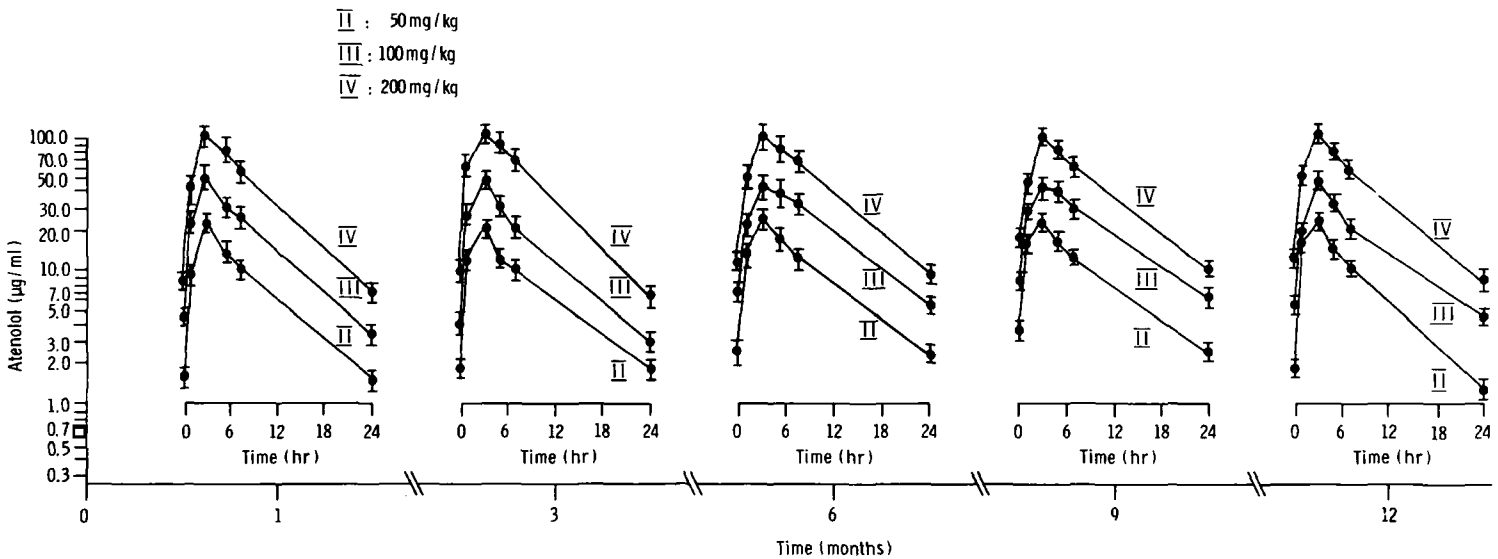


Figure 4. Levels of atenolol (µg/ml)±s.e. on whole blood of dogs dosed orally in toxicity trial TFD/140

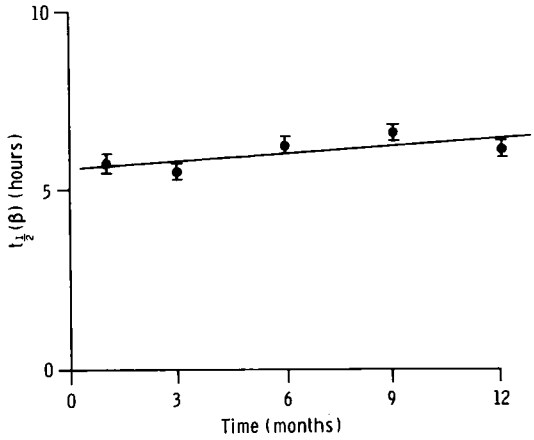


Figure 5. Average elimination phase half-lives (±s.e.) as a function of time in dogs in toxicity trial TFD/140

The areas under the blood level curves over the 24 h dosing interval were calculated using the formula

$$\text{AUC (0–24)} = (\text{AUC (0–7)}^{\text{Trapezoidal}} + \frac{(c\ (24) - c(7))}{(\beta)}$$

where $\beta = 0{\cdot}693/t_{\frac{1}{2}}$.

The average Wagner plateau level was calculated by the formula

$$\bar{c}_{\infty} = \frac{\text{AUC (24)}}{24}$$

The results from the appropriate t-tests showed that both the AUC's and c's were independent of sex. The overall plateau blood levels together with their standard errors were then evaluated and the results are illustrated in Figure 6. The dose dependency is clearly evident but there are no major changes throughout the test for the three Groups i.e. c for Groups II, III, and IV are approximately 8 μg/ml, 17 μg/ml and 40 μg/ml respectively.

This consistency in half-life, area under the curve and plateau blood levels, throughout the test is further illustrated in Figure 4 where it can be seen that the blood level patterns are very similar for the three doses at each sampling point. Furthermore the plateau blood levels are plotted in Figure 7 as a function of the dose administered. For comparison, a theoretical line was calculated using the expression:

$$\bar{c}_{\infty} = \frac{\text{Dose} \times t_{\frac{1}{2}}}{0{\cdot}693 \times 24 \times V_{\beta}}$$

where $t_{\frac{1}{2}}$ was taken as the average value from intravenous and oral dosing experiments. The volume of distribution V_{β} was taken as 160 per cent body weight and the body weight as 14·2 kg. The results in Figure 7 indicate good agreement between theory and experiment.

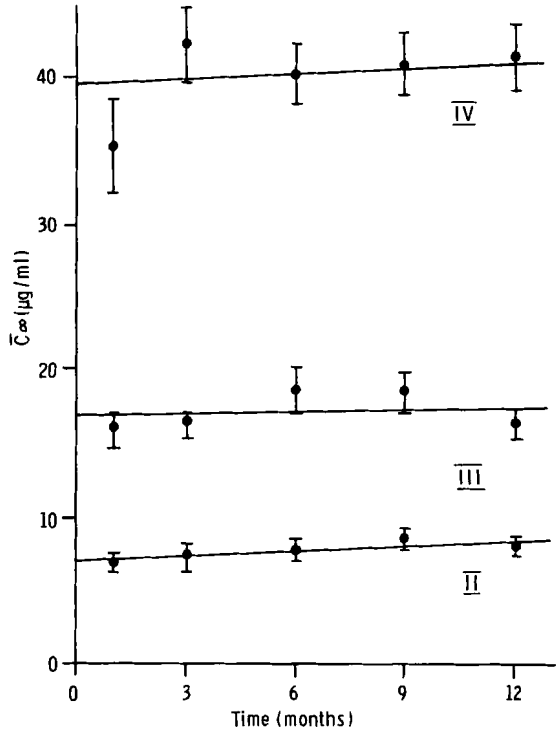


Figure 6. Average plateau levels (µg/ml) as a function of time in dogs in toxicity trial TFD/140

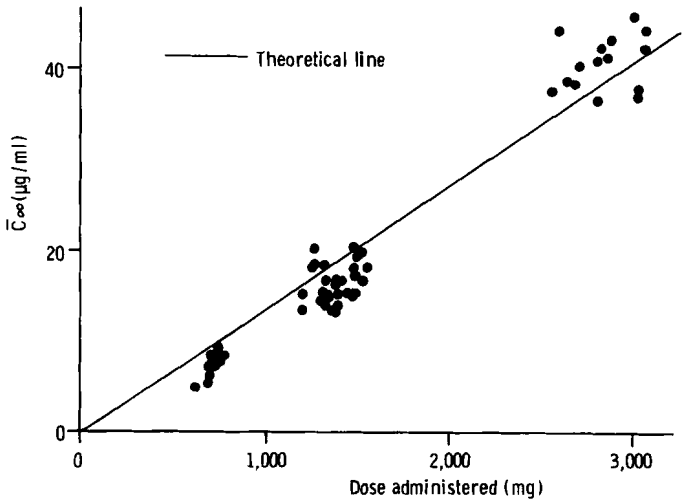


Figure 7. Wagner plateau levels (µg/ml) as a function of administered dose in dogs in toxicity trial TFD/140

CONCLUSIONS

Following intravenous dosing of atenolol to the dog at 200 mg the blood levels of parent drug were found to decay tri-exponentially with a final elimination phase half-life of about 4·5 h. The volume of distribution for the central compartment was 40 per cent body weight and whole body volume of distribution was 160 per cent body weight. The percentage urinary recovery of parent drug was 83 per cent.

Following oral dosing at 400 mg (as solution and as a clinical trial tablet) the percentage urinary recovery dropped to 65 per cent and the half-life was extended slightly to between 5 and 6 h. The peak blood levels were however very similar for the two formulations (17 and 15 μg/ml for the solution and tablet respectively) and occurred at the same time (1–2 h after dosing). The total area under the curves were similar and the values (100 $\mu g ml^{-1} h$ and 104 $\mu g ml^{-1} h$) agreed well with that anticipated on the basis of intravenous data. It was concluded that the two formulations were bioequivalent and that, following oral dosing, atenolol is almost completely absorbed with little metabolism or biliary excretion.

Following chronic oral dosing at 50, 100, and 200 mg/kg/day the systemic blood profiles were found to increase with dose at all time points throughout the study. There was no sex or dose dependency of the half-life and its value on chronic dosing was very similar to that on acute dosing as found in studies with atenolol in man.[6] The dose dependency of the area under the curves was reflected in the plateau blood levels and there was very good agreement between the experimental values and the theoretical relationship based on the acute dosing pharmacokinetic data. In accordance with the half-life there was no accumulation at any of the dose levels studied. Thus it can be concluded that atenolol obeys linear pharmacokinetics over the dose range studied. Webster *et al.*[7] also found no significant pharmacokinetic differences between chronic oral dosing and acute dosing in man.

It is worth noting that the actual values obtained in the chronic study for the atenolol blood levels (in excess of 100 μg/ml) are well in excess of the blood levels associated with normal clinical usage of atenolol[8] (peaks of about 0·7 μg/ml) and usage in renal failure patients (peak of about 4 μg/ml).[9]

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