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Research Papers

Disposition of warfarin in analbuminemic rats

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Summary

The disposition characteristics of warfarin were studied in analbuminemic rats after intravenous bolus injection of 1 and 40 mg/kg of drug to investigate the effects of plasma protein binding on drug disposition. With both doses of warfarin, total body warfarin clearance (CL) was markedly faster and its apparent volume of distribution ($V_{d\beta}$) significantly greater in the analbuminemic rats in comparison to controls. Further, the apparent elimination rate constant (β) was significantly greater and the corresponding elimination half-life ($t_{1/2\beta}$) shorter in the rats with low plasma albumin. Whole body autoradiograms demonstrated that the distribution of warfarin to the liver, skeletal muscle and brain was greater in the analbuminemic rats which was in good agreement with the larger $V_{d\beta}$ observed in this group of rats. The results suggested that the disposition characteristics of warfarin were markedly altered in the rats with low plasma albumin concentrations due to reduced plasma warfarin protein binding.

Introduction

In previous papers (Hirate et al., 1984, 1989), the disposition characteristics of salicylic acid were reported in rats with low plasma albumin concentration (analbuminemic). Following the administration of 10 mg/kg of salicylic acid, total body salicylate clearance was markedly faster and the apparent volume of distribution (V_d) significantly greater in the analbuminemic rats in comparison to controls.

It is well known that warfarin is highly plasma protein bound (> 9% in rats; Kato et al., 1986), and essentially to albumin (Kelly and O'Malley, 1979; Holford, 1986). Therefore, warfarin is an excellent model compound to study the effects of plasma albumin deficiency on drug disposition. In this paper, the disposition characteristics of warfarin in analbuminemic rats were investigated by evaluating the time course of warfarin in the plasma and whole-body autoradiography after i.v. bolus administration of warfarin following a low (1 mg/kg) and high (40 mg/kg) dose.

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Materials and Methods

Drugs and chemicals

3-α-Acetonyl [α-^{14}C] benzyl-4-hydroxycoumarin (racemic [^{14}C]warfarin) was purchased from Amersham Japan Ltd (Tokyo, Japan) and diluted with unlabelled racemic warfarin (Sigma, St. Louis, MO, U.S.A.) to prepare low (11.5 or 17.2 μCi/mg per 1.08 ml) and high dose (11.5 or 17.2 μCi/40 mg per 2.06 ml) solutions in normal saline. All other chemicals were analytical grade and were used without further purification.

Animals

Male analbuminemic (jcl : S.D. strain; Nagase et al., 1979) and control jcl : S.D. rats, 6–9 weeks old (~ 200 g) with blood albumin concentrations of approx. 4 mg/dl and 3.5 g/dl, respectively, were used. The left external jugular vein of each rat for drug administration and the collection of blood samples were cannulated under light ether anesthesia with a segment of silicone polymer tubing (1 mm i.d., 1.5 mm o.d.; Dow Corning, Tokyo, Japan) as described by Upton (1975). Because of their limited availability, only three rats per group were studied.

Plasma studies

Rats were administered 11.5 μCi/kg of [^{14}C]-warfarin at a dose of 1 or 40 mg/kg intravenously by bolus injection via the jugular vein cannula. After obtaining a blank sample, blood specimens (250 μl) were obtained at 5 and 20 min, and 1, 2, 7, 12, 24 and 36 h post-drug administration in disposable polypropylene tubes. Plasma samples (100 μl) were obtained following centrifugation at 10000 rpm for 1 min.

Plasma binding studies

A separate group of rats was given 11.5 μCi/kg of [^{14}C]warfarin at a dose of 1 or 40 mg/kg intravenously and a single blood specimen (> 3 ml) was collected from each rat at 30 min following drug administration. After separating the plasma fraction, the binding characteristics of warfarin in the plasma samples were determined by ultrafiltration at 25 ± 0.1°C using the EMIT FreeLevel Filter I (Syva, Palo Alto, CA, U.S.A.).

Urinary, fecal and expiratory excretion studies

Rats given 17.2 μCi/kg of [^{14}C]warfarin at a dose of 1 mg/kg intravenously were housed for 24 h individually in a glass metabolic cage (KN-650, Natsume Seisakusho, Tokyo, Japan) which permitted the measurement of expiratory $^{14}CO_2$ excretion as well as the collection of urine and feces. A mixture of ethanolamine and methanol (1 : 2, v/v) was used as the capturing agent for $^{14}CO_2$.

Warfarin analysis

The warfarin levels in the plasma, plasma ultrafiltrate, urine and feces were determined by liquid scintillation counting (LSC-903, Aloka, Tokyo, Japan) following extraction, and separation by thin-layer chromatography (TLC) using a slight modification of the method reported by Yacobi et al. (1974). Briefly, after extracting the sample with ethylene dichloride, TLC analysis (Kieselgel 60 F_{254}, Merck, Darmstadt, F.R.G.) was performed using a mixture of ethylene dichloride and acetone (9 : 1) as the developing solvent to separate unchanged warfarin from its metabolites. The isolated warfarin spots were scraped off the plates and suspended in 10 ml of scintillation fluid (2,5-diphenyloxazole (PPO), 5 g; 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP), 0.3 g; toluene, 700 ml; Triton X-100, 300 ml) in counting vials. The cpm values were converted to dpm using the external standard ratio method.

Whole body autoradiography studies

Rats given 17.2 μCi/kg of [^{14}C]warfarin at a dose of 1 or 40 mg/kg intravenously were killed 5 and 60 min after drug administration by immersion in a dry ice-acetone bath under anesthesia with ether. Whole body sections (40 μm) were obtained with a microtome (LKB 2250-450 MP, Bromma, Sweden) at −25°C and attached to Salotape (Hisamitsu Pharmaceutical, Tosu, Japan). After drying the whole body sections in a freeze-dryer for several days, they were placed in contact with X-ray film (No. 150, Fuji Photo Film, Tokyo, Japan) for 5 months. A densitometer (PDA-15, Konishiroku Photo, Tokyo, Japan) was used to determine the absorbance of the autoradiograms to assess the distribution patterns of radioactivity.

Data analysis

The plasma warfarin concentration-tima data were evaluated using a two-compartment open model (Gibaldi and Perrier, 1982). Eqn 1 was fitted to the plasma data by non-linear least-squares regression analysis.

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

In Eqn 1, C_p is the plasma drug concentration at time t after i.v. bolus administration, α and β are first-order hybrid disposition rate constant ($\alpha > \beta$), and A and B are the corresponding ordinate-axis intercept constants.

Total body warfarin clearance (CL) and apparent volume of distribution ($V_{d\beta}$) were determined by Eqns 2 and 3, respectively.

$$CL = \frac{\text{Dose}}{A/\alpha + B/\beta} \quad (2)$$

$$V_{d\beta} = \frac{CL}{\beta} \quad (3)$$

The apparent elimination half-life ($t_{1/2\beta}$) was estimated from Eqn 4:

$$t_{1/2\beta} = \frac{0.693}{\beta} \quad (4)$$

The data obtained in the analbuminemic and control rats were evaluated by analysis of variance and Newman-Keuls' multiple range test at the 0.05 significance level. All values are reported as means ± S.D.

Results

Plasma concentrations

The plasma warfarin concentration-time curves obtained in the analbuminemic and control rats after intravenous administration of 1 and 40 mg/kg of warfarin are presented in Fig. 1. With both doses, the plasma concentrations of warfarin obtained in the analbuminemic rats were markedly lower than the corresponding controls.

The two-compartment open model was the best-fit model to describe the data for all rats.

TABLE 1

Summary of the sum of squared deviations and AIC obtained after fitting the data for the analbuminemic rats to one- or two-compartment model equation

No. of rats	Weight	Low dose					High dose				
		No. of data points	One-compartment		Two-compartment		No. of data points	One-compartment		Two-compartment	
			s.s. [a]	AIC [b]	s.s.	AIC		s.s.	AIC	s.s.	AIC
1	1	6	0.02	−20.8	1.51×10^{-4}	−44.8	6	2.59	9.72	1.13	8.73
	$1/C$		0.08	−11.3	0.01	−19.1		0.78	2.54	0.05	−10.3
	$1/C^2$		1.87	7.76	0.07	−7.99		0.19	−6.05	2.49×10^{-3}	−28.0
2	1	6	0.04	−15.4	– [c]	–	6	23.3	22.9	–	–
	$1/C$		0.20	−5.51	5.54×10^{-3}	−23.2		1.80	7.53	0.23	−0.82
	$1/C^2$		1.41	6.06	0.45	3.19		0.49	−0.30	0.03	−12.2
3	1	5	0.04	−11.6	1.70×10^{-6}	−58.4	6	13.3	19.5	–	–
	$1/C$		0.33	−1.60	1.46×10^{-4}	−36.2		1.76	7.39	0.29	0.54
	$1/C^2$		0.53	0.81	6.21×10^{-3}	−17.4		0.20	−5.79	0.01	−17.6

[a] Sum of squared deviation.
[b] Akaike information criterion (Yamaoka et al., 1978).
[c] Not calculable.

Although the number of data points for the analbuminemic rats was only five to six because of their rapid elimination of phenytoin, the sum of squared deviations and AIC (Akaike Information Criterion, Yamaoka et al., 1978) indicated that, even in the analbuminemic rats, the two-compartment model was the best (Table 1).

Table 2 summarizes the disposition constants for warfarin observed in this study. With the 1 mg/kg dose, when compared to the controls, the total body clearance of warfarin was markedly faster (772 ± 159 vs 12.7 ± 0.4 ml/h per kg) and its apparent volume of distribution significantly greater (2.53 ± 0.54 vs 0.24 ± 0.04 l/kg) in the analbuminemic rats. Further, the apparent elimination rate constant, β, observed in the rats with low plasma albumin levels was approx. 6-fold greater than the control values, as were the apparent elimination half-life values of 2.28 ± 0.27 and 13.2 ± 1.9 h seen in the analbuminemic and control rats, respectively.

After the administration of the 40 mg/kg warfarin dose, although less pronounced, similar differences between the two groups of rats were noted for all of the disposition parameters (Table 2).

When the results obtained with the two control groups were compared, $t_{1/2\beta}$ was shorter in the group administered the 40 mg/kg warfarin dose. With the analbuminemic rats, a dose-dependent reduction in CL was noted as the warfarin dose increased.

TABLE 2

Disposition constants for warfarin obtained in control and analbuminemic rats after intravenous administration of 1 and 40 mg/kg of warfarin [a]

Parameter	1 mg/kg warfarin dose		40 mg/kg warfarin dose	
	Control	Analbuminemic	Control	Analbuminemic
CL (ml/h per kg)	12.7 ±0.4	772 [b] ±159	27.9 ±1.6	606 [b,d] ±59
$V_{d\beta}$ (l/kg)	0.24 ±0.04	2.53 [b] ±0.54	0.34 ±0.04	2.68 [b] ±1.77
β (h^{-1})	0.053 ±0.008	0.31 [b] ±0.04	0.082 ±0.005	0.28 [b] ±0.12
$t_{1/2\beta}$ (h)	13.2 ±1.9	2.28 [b] ±0.27	8.47 [c] ±0.46	2.99 [b] ±1.74

[a] Mean ± S.D., $n = 3$ rats.
[b] Significantly different from corresponding control group ($p < 0.05$).
[c] Significantly different from control group given 1 mg/kg of warfarin ($p < 0.05$).
[d] Significantly different from analbuminemic group given 1 mg/kg of warfarin ($p < 0.05$).

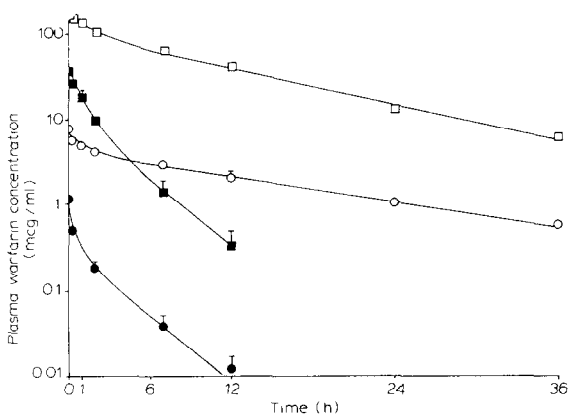

Fig. 1. Semilogarithmic graphs of the plasma warfarin concentration-time data obtained in analbuminemic (solid symbols) and control rats (open symbols) after intravenous administration of 1 (circles) and 40 mg/kg (squares) of warfarin. Each point represents the mean ± S.D. for $n = 3$ rats. The solid lines are the best-fit computer-generated curves.

Plasma protein binding

As presented in Table 3, the extent of warfarin binding in the plasma of analbuminemic rats was significantly lower than the corresponding controls, 63.6 ± 4.1 vs $98.9 \pm 0.3\%$ following the administration of 1 mg/kg of warfarin and 38.1 ± 2.0 vs $92.7 \pm 1.8\%$ for the 40 mg/kg dose. Thus, the plasma free fractions of warfarin in the analbuminemic rats were 33- and 8-fold greater than the controls after the administration of the respective doses.

In both the control and analbuminemic rats, warfarin binding was concentration-dependent, diminishing as the plasma warfarin concentration increased.

Urinary, fecal and expiratory excretion

Table 4 summarizes the cumulative amounts of total radioactivity and unchanged warfarin that were excreted in the urine, feces and expired air over a 24 h period following intravenous administration of [^{14}C]warfarin at a dose of 1 mg/kg. No differences in cumulative urinary, fecal and expiratory excretion of total radioactivity were observed between the analbuminemic and control rats. The urinary excretion of unchanged warfarin, however, was significantly greater in rats with low plasma albumin levels compared to the controls (4.6 ± 1.2 vs 1.6 ± 1.0%).

Whole body autoradiograms

Figs 2 and 3 show the whole body autoradiograms obtained 60 min after intravenous administration of 1 and 40 mg/kg of warfarin, respectively, in a representative analbuminemic and control rat. With the smaller warfarin dose, radioactivity was highest in the blood in the control rat. In the analbuminemic rat, on the other hand, the

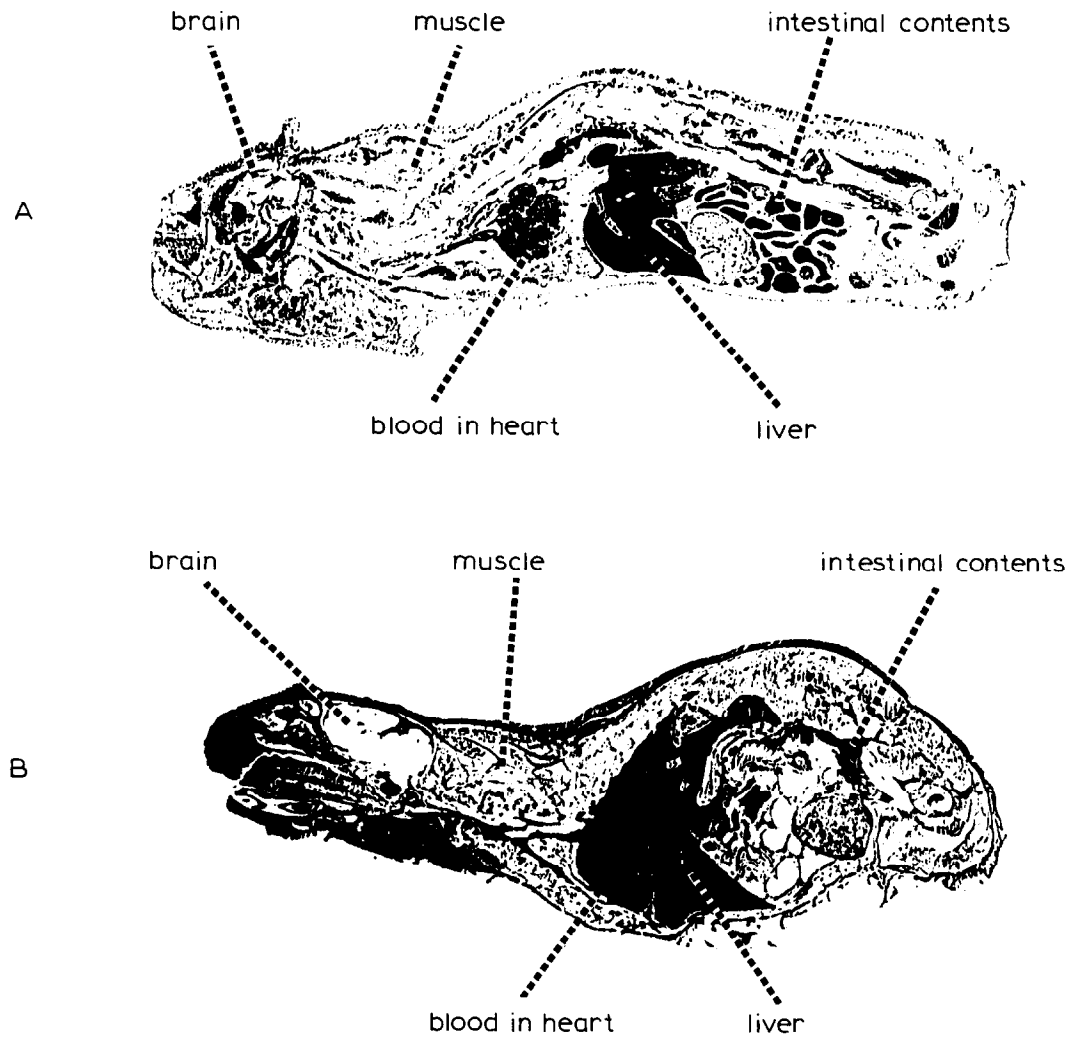

Fig. 2. Whole body autoradiograms showing the distribution of radioactivity (dark areas) at 60 min after intravenous administration of 1 mg/kg of [^{14}C]warfarin in (A) analbuminemic and (B) control rats.

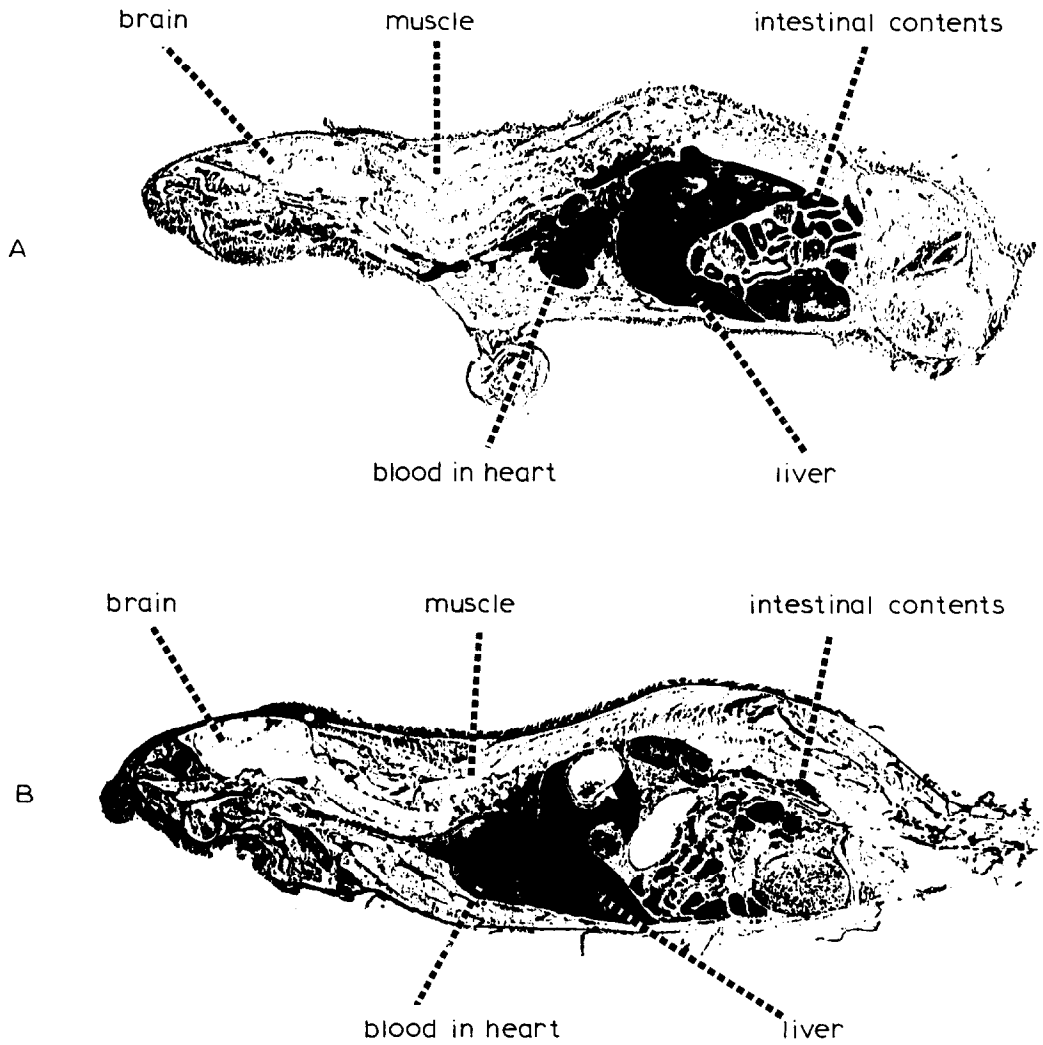

Fig. 3. Whole body autoradiograms showing the distribution of radioactivity (dark areas) at 60 min after intravenous administration of 40 mg/kg of [^{14}C]warfarin in (A) analbuminemic and (B) control rats.

highest level of radioactivity was found in the liver. Further, the radioactivity in the blood of the analbuminemic rat was considerably lower than the level seen in the blood of the control animal. The differences in the autoradiograms of the analbuminemic and control rats produced by the 40 mg/kg warfarin dose were not apparent on visual inspection (Fig. 3).

Table 5 summarizes the tissue-to-heart blood absorbance ratios for the liver, lung, skeletal muscle, and brain obtained from the respective autoradiograms at 5 and 60 min post-dose in representative analbuminemic and control rats. With the 1 mg/kg warfarin dose, the ratios for the liver, muscle and brain were larger in the analbuminemic rats at both 5 and 60 min post drug administration. As shown in Table 5, the radioactivity was particularly concentrated in the livers of the analbuminemic rats.

Although less pronounced, a similar pattern of drug distribution was noted between the two groups of rats after administration of the 40 mg/kg warfarin dose.

TABLE 3

Binding characteristics of warfarin in control and analbuminemic plasma specimens obtained 30 min after intravenous administration of 1 and 40 mg/kg of warfarin [a]

Dose (mg/kg)	Control		Analbuminemic	
	Plasma concentration (μg/ml)	Fraction bound (%)	Plasma concentration (μg/ml)	Fraction bound (%)
1	5.8 ±0.8	98.9 ±0.3	0.41 ±0.06	63.6 [b] ±4.1
40	143.5 ±15.3	92.7 [c] ±1.8	23.6 ±3.2	38.1 [b,d] ±2.0

[a] Mean ± S.D., $n = 3$ rats.
[b] Significantly different from corresponding control group ($p < 0.05$).
[c] Significantly different from control group given 1 mg/kg of warfarin ($p < 0.05$).
[d] Significantly different from analbuminemic group given 1 mg/kg of warfarin ($p < 0.05$).

Discussion

It is generally assumed that in the vascular system, only the free or unbound form of a drug is capable of undergoing distribution and elimination. Hence, any change that might occur in the degree or extent to which a drug is bound to plasma proteins has the potential to alter the drug's disposition characteristics in the body. Using analbuminemic rats and warfarin as a model compound, this study demonstrated the marked effects of plasma protein binding differences on drug disposition.

Table 3 shows that the binding of warfarin to plasma proteins was substantially lower in the analbuminemic rats in comparison to the controls which was in agreement with an earlier report for the plasma binding of warfarin in rats (Kato et al., 1986). This low plasma binding of warfarin would explain the lower plasma warfarin concentrations observed in the analbuminemic rats and associated larger $V_{d\beta}$, CL and β after both warfarin

TABLE 4

24-h urinary, fecal and expiratory excretion of total radioactivity and unchanged warfarin following intravenous administration of [^{14}C]warfarin at a dose of 1 mg/kg [a]

	Control			Analbuminemic		
	Urine	Feces	Exp. air	Urine	Feces	Exp. air
Total radioactivity (%)	34.5 ± 9.9	12.4 ± 4.0	0.11 ± 0.03	49.0 ± 13.5	23.7 ± 12.6	0.14 ± 0.08
Unchanged drug (%)	1.6 ± 1.0	6.7 ± 1.3	–	4.6 ± 1.2 [b]	7.6 ± 1.2	–

[a] Mean values ± S.D., $n = 3$ rats. [b] Significantly different from control group by Student's t-test, $p < 0.05$.

TABLE 5

Tissue-to-heart blood absorbance ratios for various tissues obtained from autoradiograms of representative analbuminemic and control rats 5 and 60 min after intravenous administration of 1 and 40 mg/kg of warfarin containing [^{14}C]warfarin

Tissue	5 min						60 min					
	1 mg/kg			40 mg/kg			1 mg/kg			40 mg/kg		
	Control	Analbuminemic	Analbuminemic/control	Control	Analbuminemic	Analbuminemic/control	Control	Analbuminemic	Analbuminemic/control	Control	Analbuminemic	Analbuminemic/control
Liver	0.81	3.17	3.91	0.70	0.98	1.40	0.82	6.27	7.65	0.81	1.17	1.44
Lung	0.79	0.65	0.82	0.79	0.73	0.92	0.82	0.45	0.55	0.74	0.46	0.62
Muscle	0.10	0.17	1.70	0.13	0.20	1.54	0.18	0.27	1.50	0.12	0.17	1.42
Brain	0.02	0.13	6.50	0.06	0.12	2.00	0.03	0.18	6.00	0.07	0.13	1.86

doses (1 and 40 mg/kg). Whole body autoradiograms and their corresponding absorbances showed that the distribution of warfarin into the liver, skeletal muscle and brain tissue was more pronounced in the analbuminemic rats (Figs 2 and 3 and Table 5). It was particularly noteworthy that the livers of the analbuminemic rats retained a remarkably high level of radioactivity 60 min after the administration of 1 mg/kg of warfarin. The greater distribution of warfarin into these selected organ and tissues in the analbuminemic rats was consistent with the larger $V_{d\beta}$ that was noted in this group of rats.

It was possible that an increase in drug metabolizing activity for warfarin may have been responsible for the greater clearances that were noted in the analbuminemic rats in this study since, as shown in Table 4, renal excretion of unchanged warfarin was a minor route of elimination in both the analbuminemic and control rats. However, since the hepatic drug metabolizing capabilities are considered to be similar between the two groups of rats (Hirate et al., 1989), diminished plasma warfarin binding rather than inherent differences in hepatic drug metabolizing activity more than likely accounted for the increased clearances observed.

Interestingly, from the binding data obtained with the plasma of the analbuminemic rats, it was apparent that warfarin binds with components other than albumin in the plasma. These interactions were also concentration-dependent (Table 3). It has been shown that in the analbuminemic strain of rats used, the decreased albumin concentration is compensated by an increase in globulin concentrations, since the total plasma protein concentration is identical for the analbuminemic and control rats (Nagase et al., 1979). Therefore, the data suggested that the plasma globulin fractions play an important role in the binding of warfarin in the plasma of the mutant rats.

The metabolism of warfarin is known to be dose-dependent (Covell et al., 1983). In previous studies (Takada and Levy, 1979, 1980) on the dose-dependent pharmacokinetics of warfarin in rats administered 0.1 or 1.0 mg/kg of drug, it was reported that the apparent volume of distribution and total plasma clearance of warfarin diminished as the dose increased. Hence, it was possible to examine this aspect in the present investigation with the two rat groups. In the rats with normal plasma albumin concentration, CL, $V_{d\beta}$ and β were similar following the administration of the 1 and 40 mg/kg warfarin doses despite the marked differences in plasma free fractions (Table 2 and 3). With analbuminemic rats, however, CL was significantly reduced as the warfarin dose increased from 1 to 40 mg/kg, suggesting dose-dependent modifications in warfarin disposition due to the capacity-limited or saturable metabolism.

In this paper, the pharmacokinetic parameters for warfarin were estimated by a linear kinetic model which was based on the assumption that the elimination rate was proportional to the total plasma concentration. Therefore, the estimated pharmacokinetic parameters might contain the probable error from the nonlinearity of warfarin disposition.

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