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Influence of Lignocaine on Plasma Protein Binding and Pharmacokinetics of Verapamil in Dogs

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Abstract—The effect of lignocaine (lidocaine) on the plasma protein binding of verapamil has been studied in-vitro and in-vivo in dogs. The binding of verapamil was ca 85%. In-vitro addition of lignocaine at therapeutic concentrations displaced verapamil from its plasma binding sites. Lignocaine in this regard was equipotent with tris(2-butoxyethyl)phosphate, suggesting an interaction at the level of α_1-acid glycoprotein binding sites. On in-vivo administration of 4 mg kg^{-1} in a bolus to dogs in which steady state concentrations of verapamil were present, the free fraction of verapamil increased transiently. During the lignocaine maintenance infusion, it then decreased to a level higher than that before administration of the local anaesthetic. The free verapamil concentrations increased suddenly upon the administration of the lignocaine loading dose, and then returned to values slightly higher than those before lignocaine. After a bolus injection of verapamil during a lignocaine infusion, the verapamil total plasma concentrations were lower than during a saline infusion, but the free concentrations were not different. The volume of distribution of verapamil was increased, whereas the blood clearance had not changed; the lignocaine infusion did not change the hepatic blood flow, as measured by indocyanine green clearance. These results show that lignocaine displaces verapamil in-vitro and in-vivo from its plasma protein binding sites, but the ensuing pharmacokinetic changes do not lead to significant changes in free verapamil concentrations.

In man verapamil is highly bound to plasma proteins and, as for many other basic drugs, both α_1-acid glycoprotein and albumin are involved (Echizen & Eichelbaum 1986). In-vitro, verapamil can be displaced from its binding sites in human plasma by drugs such as lignocaine, diazepam, propranolol and disopyramide (Young et al 1980). Recently, Chelly et al (1987) reported that, in-vitro, lignocaine (lidocaine) failed to displace verapamil from its protein binding sites in dog plasma. In contrast, we observed that lignocaine displaced verapamil from its plasma binding sites in-vivo as well as in-vitro. We report here the influence of the addition of lignocaine on the in-vitro binding of verapamil, and the results of two series of in-vivo experiments in dogs: (1) the plasma protein binding and plasma concentrations of verapamil during an intravenous infusion of verapamil, measured before and during an infusion of lignocaine, (2) the plasma protein binding and plasma concentrations of verapamil after an intravenous bolus injection of verapamil, measured during an intravenous infusion of either saline or lignocaine.

Materials and Methods

Materials

For the in-vitro studies (±)-verapamil HCl was from Sigma (Poole, UK), and [^{3}H]-verapamil HCl (70 Ci $mmol^{-1}$, radiochemical purity 99%) from New England Nuclear (Dreieich, West Germany). In-vivo (±)-verapamil HCl was given as Isoptine (amp. 5 mg/2 mL, Knoll Pharmaceuticals, Brussels, Belgium). The internal standard for the verapamil assay was 1,6-bis-(3,4 dimethophenyl)-3-methyl-aza-6-cyano-7-methyl-octane hydrochloride (D517), Knoll Pharmaceuticals (Ludwigshafen, FRG). Lignocaine HCl was given as Xylocaine (amp. 2%, Astra-Nobelpharma, Brussels, Belgium). Tris(2-butoxyethyl)phosphate (TBEP) was kindly supplied by Ega-Chemie AG (Brens, FRG). Visking membrane tubing, used for equilibrium dialysis, was purchased from Medicell International (London, UK), and indocyanine green was from Hynson, Westcott and Dunning (Baltimore, USA).

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General procedure

In-vivo, two series of experiments were performed:

Series 1. Three trained healthy dogs, 23–32 kg, were given an intravenous loading infusion of verapamil (0·015 mg kg^{-1} min^{-1} for 10 min) followed by a maintenance infusion of 0·005 mg kg^{-1} min^{-1} for 180 min. In preliminary studies this regimen resulted in steady state plasma concentrations of verapamil within the therapeutic range (Kittleson 1983). Seventy minutes after starting the verapamil administration, an intravenous loading dose of lignocaine (bolus of 4 mg kg^{-1}) was given, followed by a maintenance infusion (0·08 mg kg^{-1} min^{-1}) for 120 min as this regimen had been found to result in steady state concentrations of lignocaine within the therapeutic range (De Rick et al 1981). Heparinized blood samples for determination of the plasma protein binding and the plasma concentrations of verapamil were taken from 50 to 190 min after starting the verapamil administration.

Series 2. Three trained healthy dogs, 24–25 kg, were given verapamil as an intravenous bolus of 0·5 mg kg^{-1} twice, once during a saline infusion and once during an infusion of lignocaine given intravenously at 4 mg kg^{-1} followed by 0·08 mg kg^{-1} min^{-1}. The saline and lignocaine experiments were

separated by at least 10 days. Heparinized blood samples were taken at intervals of time up to 300 min after injection of verapamil for measurement of blood and plasma concentrations and for plasma protein binding. The clearance of indocyanine green (ICG) was measured just before the verapamil injection and at the end of the experiment. The dye was injected intravenously (0·5 mg kg^{-1}) and heparinized blood samples (6 mL) were obtained before injection and at 3 min intervals up to 18 min after injection.

For the in-vitro study of the interaction between verapamil and lignocaine or TBEP, blank blood samples were taken from the three dogs of the in-vivo series 1.

Binding assay

Protein binding of verapamil was measured by equilibrium dialysis, performed in duplicate at 25°C during 7 h in Teflon half-cells separated by a Cellophane membrane as described by Belpaire et al (1987). In control experiments no protein was added to ascertain that 7 h were sufficient for equilibration of the ligand. One compartment contained phosphate buffer (0·3 mL, 0·15 M, pH 7·4) in which verapamil was dissolved, and the other contained plasma. For the study of the in-vitro interaction, verapamil was added to the buffer compartment in a concentration, before dialysis, of 70 ng mL^{-1}, containing 0·125 μCi mL^{-1} [^{3}H]verapamil; lignocaine or TBEP was added to the plasma compartment before dialysis in concentrations ranging from 1 to 50 μg mL^{-1} for lignocaine and from 3 to 100 μg mL^{-1} for TBEP. For the in-vivo study, samples obtained from the treated dogs were dialysed against buffer containing 0·125 μCi mL^{-1} [^{3}H]-verapamil.

For both the in-vivo and in-vitro studies, plasma pH before dialysis was adjusted to 7·4 with HCl (1 M). The pH increased maximally with 0·1 pH unit over the dialysis period, and such a small change had only a negligible effect on verapamil binding. At the end of dialysis, 100 μL aliquots from each compartment were added to scintillation vials containing 4 mL Aqualuma Plus (Lumac, Schaesberg, The Netherlands), and radioactivity was measured by scintillation counting in a Packard Tricarb Scintillation Spectrometer. The free fraction was calculated from the ratio, at equilibrium, of the number of disintegrations per minute in buffer to that in plasma.

Drug assays

Verapamil concentrations were measured by high-performance liquid chromatography (HPLC) using a Spherisorb 5 ODS column (25 cm × 4·6 mm i.d.) (Chrompack, Belgium) and a mixture of 0·04 M sodium phosphate buffer (pH 3) with 0·07 M triethylamine–methanol (55:45, v/v) as mobile phase, with a flow of 1 mL min^{-1} at 50°C. The fluorometer (Schoeffel 970, USA) was set at an excitation wavelength of 230 nm, with an emission cutoff filter of 305 nm. The retention times of verapamil and of the internal standard (D517) were 7·5 and 6·5 min, respectively. Verapamil and its internal standard (D517) were extracted from alkalinized plasma or blood (pH 13) with a mixture of heptane–butanol (94:4, v/v) and re-extracted from the organic phase into 0·1 M sulphuric acid. The between-run coefficient of variation was 1·2% at a concentration of 50 ng mL^{-1} (n = 5).

Lignocaine concentrations in plasma were measured with HPLC as described previously (De Rick et al 1987). ICG concentrations were measured directly in plasma by spectrometry at a wavelength of 800 nm (Vu Van et al 1983). Because ICG is unstable in plasma, the assay was done within 3 h of sampling. The log absorbance vs time data were fitted to a straight line by a least-square regression analysis and the ICG half-life was determined from the slope.

Pharmacokinetic calculations

Plasma verapamil concentrations-time course data of individual animals from the intravenous bolus study (second series) were fitted to the equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ (Gibaldi & Perrier 1982) using a non-linear least-square regression computer program Multi (Yamaoka et al 1981). The systemic plasma clearance CLs was calculated from CLs = dose/$(A/\alpha + B/\beta)$. The volume of distribution ($V_{d\beta}$) was calculated as $V_{d\beta} = CLs \cdot \beta$ and the area under the plasma concentration-time curve (AUC) was calculated as $AUC = A/\alpha + B/\beta$ (Gibaldi & Perrier 1982).

The verapamil blood to plasma concentration ratio (B/P) was determined for each sample and the mean value for each dog was calculated and used to determine the blood clearance (CL_{bl}) from $CL_{bl} = CL_{pl}/(B/P)$.

ICG plasma clearance was calculated from dose divided by $AUC_{0->\infty}$ and this was converted to blood clearance by the equation CL_B = plasma clearance × 1/(1-haematocrit).

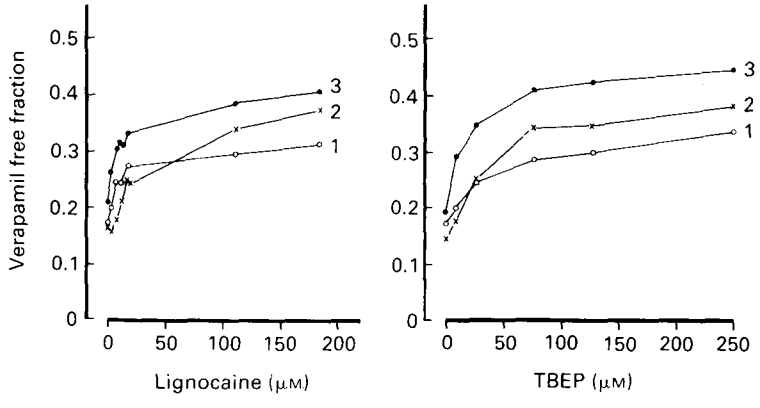

FIG. 1. Percentage free verapamil in plasma of three dogs as a function of the concentration, before dialysis, of lignocaine (left panel) and TBEP (right panel). Total verapamil concentration before dialysis was 70 ng mL^{-1}.

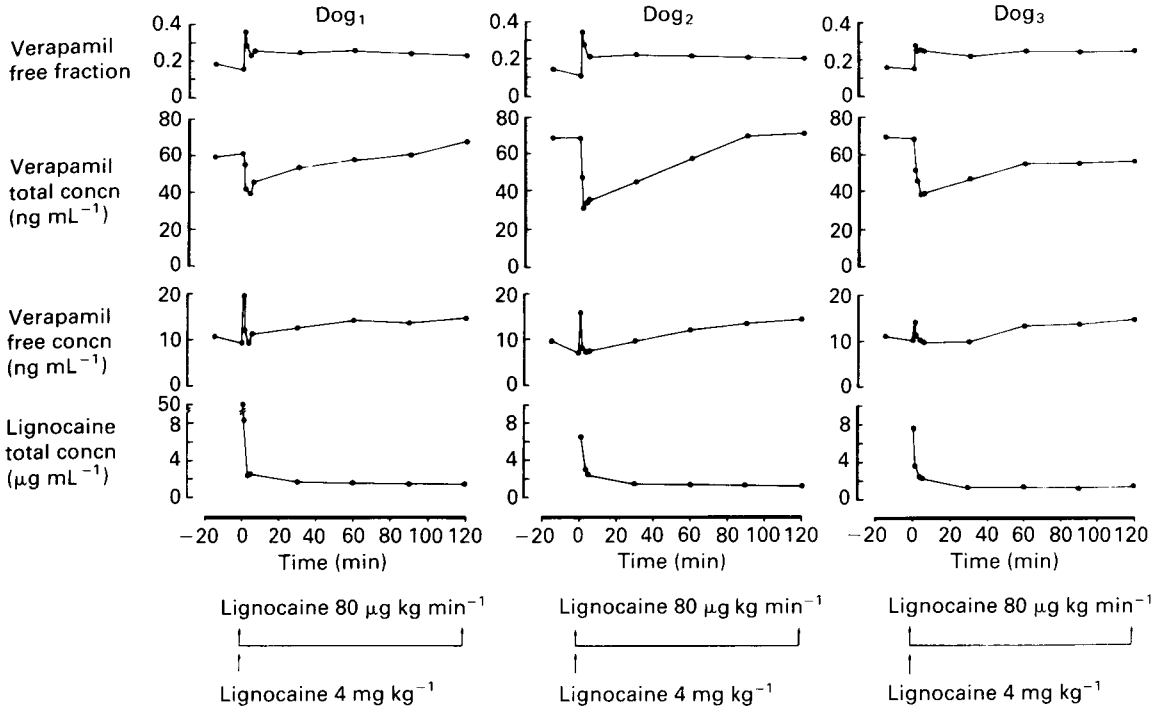

FIG. 2. Effect of an intravenous lignocaine administration (4 mg kg^{-1} as a bolus followed by 0·08 mg kg^{-1} min^{-1} over 120 min) on percentage free verapamil and on total and free steady state verapamil plasma concentrations in three dogs. Verapamil steady state concentrations were obtained by giving intravenously a loading dose of 0·015 mg kg^{-1} min^{-1} over 10 min, followed by 0·005 mg kg^{-1} min^{-1} over 180 min. Plasma lignocaine concentrations as a function of time are also shown. Time 0 corresponds to the start of the lignocaine infusion.

Results

Influence of in-vitro addition of lignocaine on the plasma binding of verapamil

Fig. 1 shows the verapamil free fraction in the presence of increasing concentrations of lignocaine in the plasma of the three dogs. Lignocaine displaced verapamil in a dose-dependent manner, as did TBEP; TBEP and lignocaine were approximately equipotent in this regard.

Influence of in-vivo administration of lignocaine on plasma binding and steady state concentrations of verapamil

Fig. 2 shows the free fraction and the total and free plasma concentrations of verapamil during steady state, before and during administration of lignocaine to the three dogs. Immediately after injection of the loading dose of lignocaine, the free fraction of verapamil more than doubled. This was followed by a rapid decrease to a value which was only about 5% higher than the value found before lignocaine admini-

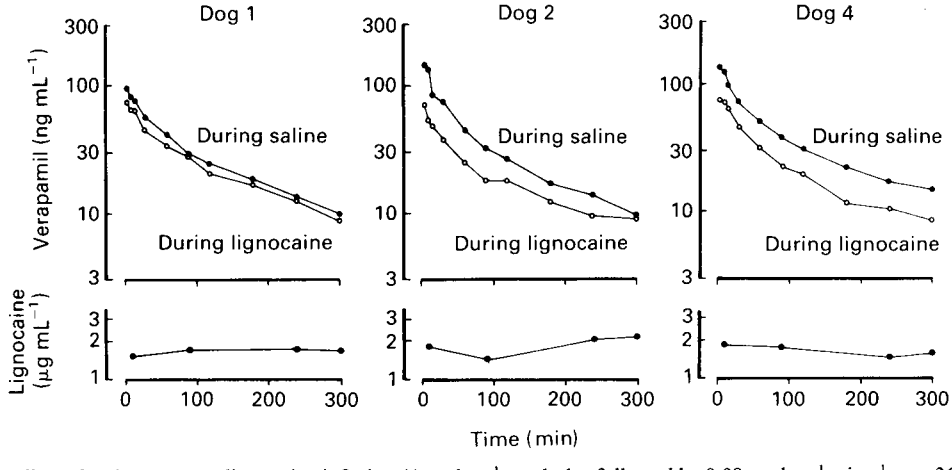

FIG. 3. Effect of an intravenous lignocaine infusion (4 mg kg^{-1} as a bolus followed by 0·08 mg kg^{-1} min^{-1} over 360 min) or a saline infusion, on plasma concentrations of verapamil given as an intravenous bolus injection (0·5 mg kg^{-1}).

Table 1. Pharmacokinetic parameters of verapamil after intravenous administration and ICG clearance in three dogs during intravenous infusion of either saline or lignocaine (L).

	Dog 1		Dog 2		Dog 3	
	Saline	L	Saline	L	Saline	L
Free fraction	0·18	0·25	0·19	0·26	0·16	0·24
Blood/plasma ratio	1·07	1·22	0·72	1·04	0·83	1·10
AUC_{pl} ($\mu g\ mL^{-1}\ min$)	10·6	9·2	12·0	8·6	14·9	9·9
AUC_{bl} ($\mu g\ mL^{-1}\ min$)	11·0	10·1	8·6	7·0	11·0	10·6
CL_{pl} ($mL\ min^{-1}\ kg^{-1}$)	47·4	54·6	41·8	57·5	33·5	50·6
CL_{bl} ($mL\ min^{-1}\ kg^{-1}$)	43·5	44·8	58·0	58·1	39·8	45·2
V_d ($L\ kg^{-1}$)	9·8	11·1	7·9	18·0	8·4	18·8
$t_{\frac{1}{2}}$ (min)	142	118	128	208	169	255
ICG CL_{bl} ($mL\ min^{-1}$)						
First	325	260	589	465	380	515
Second	378	278	528	443	495	475

CL_{pl} plasma clearance; CL_{bl} blood clearance; V_d volume of distribution; $t_{\frac{1}{2}}$ half life; ICG CL_{bl} indocyanine green blood clearance.

stration. Lignocaine induced a transient decrease in verapamil total plasma concentrations of about 50%, and at the same time an increase in free concentrations of about 70%. The total verapamil concentrations returned slowly to base line values during the 2 h of lignocaine infusion; the free concentrations, after the transient increase due to the administration of the lignocaine loading dose, returned rapidly to base line values; they then increased again slowly up to 2 h after lignocaine infusion to values which were only slightly higher than the base line values.

The plasma concentrations of lignocaine are also shown in Fig. 2. After the loading dose, in the three dogs the plasma concentrations decreased rapidly and after about 30 mins, steady state concentrations of approximately 1·5 $\mu g\ mL^{-1}$ were obtained.

Influence of in-vivo administration of lignocaine on plasma binding and concentrations of verapamil given as a bolus

Fig. 3 shows the plasma concentrations of verapamil after a bolus injection, during either a saline or a lignocaine infusion in the three dogs. Lignocaine steady state concentrations are also shown.

During lignocaine infusion, the plasma concentrations of verapamil were lower than during saline infusion. In Table 1 the pharmacokinetic parameters of verapamil and the ICG clearance during saline and lignocaine infusion are summarized. During the infusion into the three dogs, the free fraction of verapamil and its blood to plasma concentration ratio increased. The AUC's for verapamil in plasma are markedly lower during lignocaine than during saline injection. The plasma clearance and the volume of distribution were increased.

The AUC's for verapamil in blood and the blood clearance were not markedly changed during lignocaine infusion nor was there any difference in ICG clearances between the saline and lignocaine infusions.

Discussion

In the three dogs the in-vitro binding of verapamil in plasma varied between 85 and 90%. These values are slightly lower than those reported by others (90·7%, Keefe et al 1981; 93·9%, Chelly et al 1987), in the same species. In man, both verapamil and lignocaine bind to α_1-acid glycoprotein (AGP) (Routledge et al 1980; McGowan et al 1983; Gillis et al 1985). In dogs, we previously showed that lignocaine binds to α_1-AGP (De Rick et al 1987), and we found that this is also true for verapamil. Indeed TBEP, a specific inhibitor of α_1-AGP binding, displaced verapamil from its binding sites. Our in-vitro study showed a concentration-dependent displacement of verapamil by lignocaine at therapeutic concentrations of both drugs corresponding with the results of Young et al (1980) in man. In contrast, Chelly et al (1987), likewise in an in-vitro study, did not find this in dogs. Indeed, Chelly et al (1987) dialysed 1 mL of serum containing lignocaine and verapamil, against 8 mL of buffer. This led to a dilution, with a low total concentration of lignocaine, so that its displacing effect might be much less pronounced. In our experiments, equal volumes of plasma and buffer were used for the equilibrium dialysis.

In-vivo administration of lignocaine increased the free fraction of verapamil in both series of experiments, confirming our in-vitro results. At the same time there was a transient, but important, decrease of total verapamil plasma concentrations, which then increased again to the values seen before administration of lignocaine, as shown in our first study. The free verapamil concentrations increased transiently and then stabilized at values which were slightly higher than those before administration of lignocaine. The transient changes of free fraction and of total and free concentrations of verapamil immediately after a bolus injection of lignocaine, were also found by Chelly et al (1987). As those authors found, however, no in-vitro interaction between the two drugs, they explained their results by an increase of the volume of distribution and the intercompartmental clearance of verapamil: the latter was attributed by those authors to an increased pulmonary blood flow. That in our experiments, verapamil free concentrations rose during lignocaine infusion towards levels higher than those before lignocaine, can be explained by the increase in the free fraction, where for a high extraction drug free steady state concentrations are indeed proportional to the free fraction.

The return of total verapamil concentrations to pre-lignocaine values, is also to be expected. Lignocaine treatment has an influence on the calculated pharmacokinetic parameters of verapamil as shown in study 2. The volume of distribution was increased, as also found by Chelly et al (1987). The increase in volume of distribution can be explained, at least in part, by a decrease in the free fraction of verapamil. The total blood clearance of verapamil and the liver blood flow, as measured by the ICG clearance, varied little during the lignocaine treatment. Our results are in keeping with the hypothesis that the hepatic clearance of a high extraction drug is mostly dependent on changes in liver blood flow, and not dependent on changes in free fraction (Wilkinson & Shand 1975). The plasma clearance, however, increased more than the blood clearance during lignocaine treatment, but this can be explained by a change in the blood-plasma concentration ratio of verapamil.

Our results show clearly that in-vivo as well as in-vitro lignocaine displaces verapamil from plasma protein sites, most probably from α_1-AGP. The decrease in total concentrations of verapamil seen after lignocaine administration by Chelly et al (1987) and by us, can therefore, at least in part, be explained by a protein binding interaction. However, the fact that there is no important change in free plasma concentration suggest that, on this basis, dose adjustment is not necessary.

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