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Effects of nicorandil and verapamil, antianginal agents, on plasma digoxin concentrations in rats and dogs

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The effects of equihypotensive doses of nicorandil and verapamil on plasma digoxin concentrations have been assessed in rats and dogs. In a single digoxin dose study, digoxin (1 mg kg^{-1}) alone, or in combination with nicorandil (5 mg kg^{-1}) or verapamil (25 mg kg^{-1}) was given orally to rats. When given chronically to rats, a single dose of digoxin (1 mg kg^{-1}) orally for 7 consecutive days was followed, on day 8, by digoxin alone, or together with nicorandil (5 mg kg^{-1}) or verapamil (25 mg kg^{-1}). In dogs, a loading dose of digoxin (50 μg kg^{-1}) was given orally on day 1, then 25 μg kg^{-1} was administered for the following 6 days. On day 8, digoxin (50 μg kg^{-1}) was given with nicorandil (5 mg kg^{-1}) or verapamil (20 mg kg^{-1}). In rats, the AUC_{0-24} and C_{max} of plasma digoxin were enhanced significantly by coadministration of verapamil, but not by nicorandil. In dogs, verapamil significantly increased the C_{max} of plasma digoxin, but not the AUC. Nicorandil had no effect on either parameter.

It is well known that quinidine (Ejvinsson 1978; Leahey et al 1978) and verapamil (Pedersen et al 1981; Klein et al 1982; Belz et al 1983; Zatuchni 1984), taken during digoxin therapy, result in higher serum levels and earlier peak concentrations of digoxin than when digoxin is taken alone, thereby increasing the risk of digoxin overdosing. Clinical studies have therefore focussed on the occurrence of drug interaction between digoxin and some cardiovascular drugs including antiarrhythmic agents and Ca^{2+}-antagonists. Nicorandil, a recently developed antianginal drug, is increasingly used in the clinic for patients not only with vasospastic angina but also with effort angina (Sakai et al 1983b). Although investigations have helped to clarify its pharmacological profile (Uchida et al 1978; Taira et al 1979; Sakai et al 1981, 1983a), and to prove its therapeutic benefit to patients with angina (Sakai et al 1983b), there have been no reports of a pharmacokinetic interaction between digoxin and nicorandil.

The present study was undertaken to examine the effect of nicorandil, compared with verapamil, on plasma digoxin concentrations in rats and dogs, and to predict whether a single dose of nicorandil affects the plasma digoxin level when coadministered with oral digoxin.

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METHODS

Systemic blood pressure (SBP) and heart rate (HR) measurements

Rats. Male Sprague-Dawley rats, 350 to 380 g, were divided into 3 groups (n = 4): Group I was treated with 3% arabic gum solution (control), Group II with nicorandil (5 mg kg^{-1}) and Group III with verapamil (25 mg kg^{-1}). The animals were fasted overnight before the experiment, but had free access to tap water. The drugs were suspended in 3% arabic gum solution, and administered by gavage in a volume of 1·0 mL. Before SBP and HR measurements, rats were prewarmed in a heated chamber (about 40 °C) for 10 min, and then the systolic SBP and HR were measured indirectly with a tail-cuff plethysmograph (Narco Bio-System, Inc., Texas). All rats were conditioned to the SBP and HR procedures for 2 to 3 days, and then the parameters were determined before and at 15, 30 min, and 1, 2, 3, and 6 h after dosing.

Dogs. Labrador retrievers of either sex, 25–27 kg (n = 4), were fitted, aseptically and under pentobarbitone anaesthesia (30 mg kg^{-1}), with an indwelling catheter (PE 50) in the femoral artery so that the tip lay in the ascending aorta. The catheter, filled with sodium heparin (1000 u mL^{-1}) and flushed periodically, was arranged to exit high on the back of the animal. The dogs were trained to lie quietly on their side on a padded table for subsequent monitor-

ing. They each wore a jacket and a collar to protect the catheter from damage. To measure SBP and HR, the catheter was connected to a Nihon Kohden pressure transducer (MPU-0·5). The HR was determined with a Nihon Kohden heart rate counter (AT-600G). All recordings were made continuously on a chart by using a Watanabe Linearcorder (WR-3101). The animals were fasted overnight before the experiment, but had free access to tap water. The effects of drugs administered in gelatin capsules were examined for 6 h following dosing. Animals were dosed once a week.

Pharmacokinetics

Male Sprague-Dawley rats (340–380 g) and beagle dogs of either sex (9–10 kg) were used.

Experimental protocol

Single digoxin dose study. Rats, fasted overnight, but having free access to tap water were divided into 3 groups (n = 10). Group I was treated with digoxin (1 mg kg^{-1}) alone, Group II simultaneously with digoxin and nicorandil (5 mg kg^{-1}), and Group III simultaneously with digoxin and verapamil (25 mg kg^{-1}). Immediately before dosing, blood samples (0·15 mL) were withdrawn from the tail vein into heparinized syringes, and centrifuged at 3000 rev min^{-1} for 10 min with a Hitachi Refrigerated Centrifuge (05PR-22). Plasma was separated, transferred to test tubes and frozen at −20 °C until assayed (within 2 days). Venous blood samples (0·15 mL) were withdrawn 1, 2, 4, 6, and 24 h after oral dosing. The drugs were suspended in 3% arabic gum solution, and given by gavage in a volume of 1·0 mL.

Chronic digoxin dose study. Rats received digoxin (1 mg kg^{-1}) orally, once a day (between 0900 and 1000 h) for 7 days. Immediately before dosing, blood samples (0·15 mL) were removed from the tail vein, using heparinized syringes, for digoxin determination. The animals were fasted overnight from the evening on day 7, but had free access to tap water. On day 8, the animals were divided into 3 groups (n = 10). Group I was treated with digoxin (1 mg kg^{-1}) alone, Group II with digoxin and nicorandil (5 mg kg^{-1}), and Group III with digoxin and verapamil (25 mg kg^{-1}). Venous blood samples (0·15 mL) were withdrawn before drug treatment. Thereafter, blood samples (0·15 mL) were withdrawn from the tail vein into heparinized syringes, 1, 2, 4, 6 and 24 h after oral dosing.

Eight dogs were fasted overnight. On day 1, blood samples (1 mL) were withdrawn from the cephalic vein with heparinized syringes. At 0900 h, 50 μg kg^{-1} digoxin was given orally as a loading dose, and blood samples (1 mL) withdrawn 0·5, 1, 2, 4, 6, 8 and 24 h after dosing (served as controls). Food was given at 1500h. Digoxin was suspended in 3% arabic gum solution, and administered in gelatin capsules. As signs of toxicity such as vomiting, diarrhoea, tiredness and dizziness appeared with the 50 μg kg^{-1} digoxin dose, maintenance was with 25 μg kg^{-1} once a day (0900h) until the 6th day. Before dosing, blood samples were taken for analysis of plasma digoxin concentrations. The animals were fasted overnight from the evening on day 7, but had free access to tap water. On day 8, the dogs were divided into two groups (n = 4). Group I was treated concurrently with digoxin (50 μg kg^{-1}) and nicorandil (5 mg kg^{-1}), and Group II treated with digoxin (50 μg kg^{-1}) and verapamil (20 mg kg^{-1}). Venous blood samples (1 mL) were taken, and then (at 0900h) the combination of digoxin and nicorandil or verapamil was given orally in gelatin capsules. Thereafter, blood samples (1 mL) were withdrawn from the cephalic vein into heparinized syringes 0·5, 1, 2, 4, 6, 8 and 24 h after dosing, and plasma prepared and stored as previously.

Bioanalysis

Plasma digoxin concentrations were measured in duplicate using a radioimmunoassay kit (digoxin ^{125}I kit, Midori-juji, Japan). Radioactivity was determined by means of an Aloka γ counter (model 600). Plasma samples from rats (but not dogs) were diluted 10 times with 0·9% saline, then, 0·1 mL of each sample was used for assay. The area under the plasma concentration time curve (AUC_{0-24}) was determined planimetrically. Nicorandil and verapamil at 10 mg L^{-1} in 0·9% saline did not interfere.

The drugs used were: digoxin standard (distributed by National Institute of Hygiene, Japan), nicorandil, *N*-2-(hydroxyethyl)nicotinamide nitrate (ester) (mol. wt 211·18, $C_8H_9N_3O_4$, synthesized in our Research Laboratories) and verapamil hydrochloride (extracted from commercially available tablets) (Eisai). Values in the text are represented as means ± s.e. Significant differences between mean values were estimated by using Student's *t*-test; $P < 0·05$ was considered significant.

RESULTS

Effects of nicorandil and verapamil on systemic blood pressure (SBP) and heart rate (HR)

Basal values in each parameter are shown in Table 1. Stable conditions were maintained over 6 h following vehicle administration in rats (Fig. 1A) and dogs (Fig. 1B).

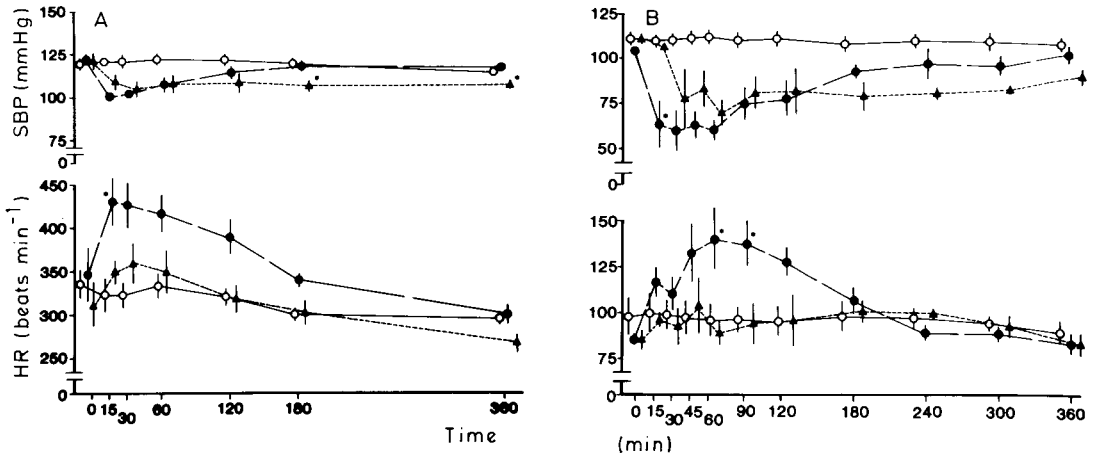

FIG. 1. Time course of the effects of nicorandil (5 mg kg^{-1}, ●) and verapamil (25 mg kg^{-1} in rats and 20 mg kg^{-1} in dogs, ▲) compared with vehicle alone (○) given orally on the systemic blood pressure (SBP) and heart rate (HR) in conscious rat (A) and dog (B). The vertical bars represent means ± s.e. of 4 observations (n = 4). *$P < 0.05$ compared with the verapamil-treated group at each time point.

Table 1. Basal values of systemic blood pressure (SBP) and heart rate (HR).

	Vehicle	Nicorandil	Verapamil
Rats			
Systolic SBP (mmHg)	118·8 ± 3·0	122·5 ± 2·5	121·5 ± 4·8
HR (min^{-1})	335·0 ± 16·7	348·0 ± 31·4	312·0 ± 23·0
Dogs			
Mean SBP (mmHg)	110·5 ± 3·5	104·0 ± 2·4	110·8 ± 3·5
HR (min^{-1})	97·5 ± 10·1	86·3 ± 2·2	85·0 ± 5·4

Values represented are means ± s.e. of 4 observations (n = 4). The values from each group demonstrated no significant differences relative to the corresponding values from the vehicle-treated group.

Rats. Nicorandil (5 mg kg^{-1}) and verapamil (25 mg kg^{-1}) both produced a significant decrease in systolic SBP (Fig. 1A) to a comparable extent (peak decrease in systolic SBP, −22·6 ± 1·4 mmHg for nicorandil and −19·0 ± 3·7 mmHg for verapamil; $P > 0.05$; each n = 4). Verapamil had a greater duration of action on SBP than nicorandil. Unlike verapamil, nicorandil markedly increased HR.

Dogs. The results were qualitatively similar to those seen in rats (Fig. 1B). Oral nicorandil (5 mg kg^{-1}) and verapamil (20 mg kg^{-1}) markedly and comparably decreased SBP (peak decrease in mean SBP, −55·0 ± 1·4 mmHg for nicorandil and −60·8 ± 3·5 mmHg for verapamil; $P > 0.05$; each n = 4). A marked increase in HR was observed in the nicorandil-treated group, whereas only a slight change in HR occurred in the verapamil-treated group.

Effects of nicorandil or verapamil on plasma digoxin concentrations

Single digoxin dose study

Rats. Fig. 2 shows the digoxin plasma profiles for the groups treated orally with digoxin (1 mg kg^{-1}) alone, and in combination with nicorandil (5 mg kg^{-1}) or verapamil (25 mg kg^{-1}). The area under the plasma concentration time curve (AUC), as well as the peak plasma concentration (C_{max}) of digoxin, was significantly greater when the drug was given with verapamil than when given alone (Table 2). On the other

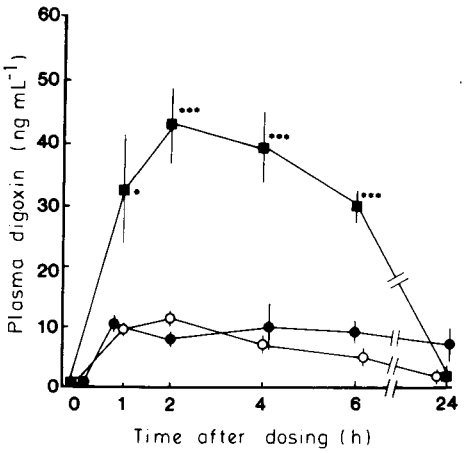

FIG. 2. Plasma digoxin concentrations in single digoxin dose study in rats. The vertical bars represent mean ± s.e. of 10 observations (n = 10). Digoxin (1 mg kg^{-1}) alone, ○; digoxin plus nicorandil (5 mg kg^{-1}), ●; digoxin plus verapamil (25 mg kg^{-1}), ■. *$P < 0.05$, ***$P < 0.001$ vs the corresponding value from the group treated with digoxin alone.

Table 2. Effects of nicorandil and verapamil on digoxin kinetics in rats.

	Single digoxin dose study (n = 10)		Chronic digoxin dose study (n = 10)	
	C_{max} (ng mL^{-1})	AUC_{0-24} (ng mL^{-1} h)	C_{max} (ng mL^{-1})	AUC_{0-24} (ng mL^{-1} h)
Digoxin 1 mg kg^{-1} alone	12·2 ± 1·0	125·6 ± 14·2	19·1 ± 3·2	194·6 ± 57·2
Digoxin with nicorandil, 5 mg kg^{-1}	13·1 ± 1·6	189·3 ± 31·4	17·4 ± 2·8	190·2 ± 41·5
Digoxin with verapamil, 25 mg kg^{-1}	47·6 ± 5·8***	486·7 ± 46·1***	79·4 ± 8·8***,†	889·6 ± 139·3***,†

Values represented are means ± s.e.
***$P < 0·001$ compared with digoxin alone; †$P < 0·001$ compared with the corresponding values obtained from single digoxin dose study.

hand, the coadministration of nicorandil did not significantly affect the plasma digoxin concentrations.

Chronic digoxin dose study

Rats. Based on all 30 animals, predose plasma digoxin concentrations were 0·74 ± 0·10 ng mL^{-1} before the treatment, 2·25 ± 0·21 ng mL^{-1} on day 2, and 2·07 ± 0·20 ng mL^{-1} on day 5. No significant difference was observed between the corresponding values on day 2 and day 5. Fig. 3 represents the plasma digoxin concentration curves for the groups treated orally with digoxin (1 mg kg^{-1}) alone, and together with nicorandil (5 mg kg^{-1}) or verapamil (25 mg kg^{-1}). The plasma concentrations of digoxin attained a peak (C_{max}) approximately 1–2 h after dosing, and decreased progressively, approaching predose values 24 h after dosing. The AUC and C_{max} of digoxin were significantly greater when digoxin was given with verapamil than alone. Furthermore, as noted in Table 2, the AUC and C_{max} of digoxin, based upon the chronic dosing with verapamil, were significantly higher than those in the single digoxin dose study, even though there were no significant differences between mean values of the AUC or C_{max} after single and chronic doses of digoxin alone. On the other hand, the coadministration of nicorandil, unlike verapamil, had no influence on plasma digoxin concentrations.

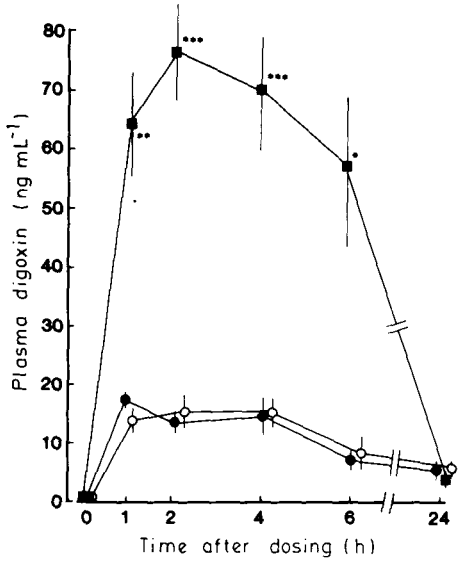

FIG. 3. Plasma digoxin concentrations in chronic digoxin dose study in rats. The vertical bars represent mean ± s.e. of 10 observations (n = 10). *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ vs the corresponding value from the group treated with digoxin alone. Symbols as in Fig. 2.

Dogs. Based on 8 animals, predose plasma digoxin concentrations were 0·56 ± 0·06 ng mL^{-1} on day 4 and 0·44 ± 0·04 ng mL^{-1} on day 6; $P > 0·05$. As seen from Fig. 4, the C_{max} of digoxin was significantly higher when digoxin was given with verapamil than when given alone, even though the AUC of digoxin was not significantly affected by the coadministration of verapamil. On the other hand, no significant influence on plasma digoxin levels was observed with the coadministration of nicorandil.

DISCUSSION

We have shown that in rats and dogs nicorandil did not affect plasma digoxin concentrations, whereas verapamil significantly enhanced them. Recently, Zatuchni (1984) reported two patients in whom combined treatment with verapamil and digoxin resulted in death. Thus, our results with verapamil are in agreement with clinical findings (Pedersen et al 1981; Klein et al 1982; Belz et al 1983).

We found that plasma digoxin concentrations after the chronic dosing tended to be relatively higher than those after the single dose, even though no significant differences were observed in the parameters measured. On the other hand, when digoxin was given with verapamil, both AUC and C_{max} of digoxin

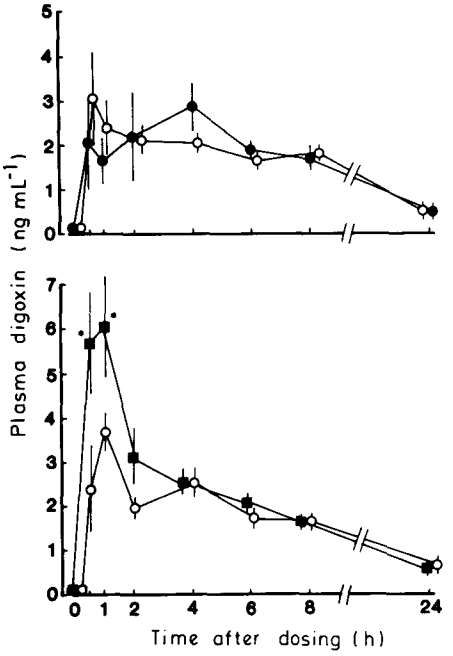

FIG. 4. Plasma digoxin concentrations in chronic digoxin dose study in dogs. Digoxin (50 μg kg^{-1}) alone ○; digoxin plus nicorandil (5 mg kg^{-1}) ●; digoxin plus verapamil (20 mg kg^{-1}) ■. The vertical bars represent mean ± s.e. of 4 observations (n = 4). *$P < 0.05$ vs the corresponding control value (see 'Methods' section).

given chronically were significantly larger than those in the single dose digoxin study. Nicorandil had no significant effect on the pharmacokinetics of digoxin.

In the present study, approximately equihypotensive effects are found with a dose of verapamil only 5 times greater than that of nicorandil (25 vs 5 mg kg^{-1}). This is in contrast to clinical practice where the normal starting doses of nicorandil (Sakai et al 1983b) and verapamil (Francis & Cohn 1985) are 5 and 80 mg, respectively, orally every 8 h, with a ratio of 1 : 16 based on weight. The lack of any effect of nicorandil on digoxin plasma levels in the study is therefore not attributable to insufficient dosing.

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