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Effects of AN-132 and quinidine, antiarrhythmic agents, on plasma digoxin concentrations in rats

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Abstract—The effects of AN-132, 3-(diisopropylaminoethylamino)-2′,6′-dimethylpropionanilide·$2H_3PO_4$, on chloroform-induced arrhythmias and plasma digoxin concentrations have been compared with those of quinidine in rats. AN-132 (0·01–3 mg kg^{-1}) administered orally significantly inhibited the incidence of cardiac arrhythmias in a dose-related fashion. A single dose of digoxin (1 mg kg^{-1}) given orally for 7 consecutive days was followed, on day 8, orally by digoxin alone, or together with AN-132 (50, 100 and 200 mg kg^{-1}) or quinidine (25 and 50 mg kg^{-1}). The AUC_{0-24} and C_{max} of plasma digoxin were enhanced significantly by co-administration of quinidine, but not by AN-132.

Although use of antiarrhythmic drugs such as quinidine during maintenance digoxin therapy has now become common in patients with cardiac disease, severe adverse effects have often been reported (Bigger & Hoffman 1985). Quinidine and some other antiarrhythmics are known to alter the pharmacokinetics of digoxin, and increase plasma/serum digoxin levels, heightening the risk of adverse reactions (Ejvinson 1978; Leahey et al 1978; Chen & Friedman 1980; Weeks et al 1986). AN-132 is a novel diamine derivative which is under development as an orally efficacious antiarrhythmic drug, and its potent antiarrhythmic activity has been evidenced in several animal models (Sakai et al 1987).

The aim of the present study was to examine the antiarrhythmic effect of AN-132, and compared with quinidine, its effect on digoxin pharmacokinetics in rats. A further aim was to predict whether a single dose of AN-132 affects the plasma digoxin level when co-administered with oral digoxin.

Methods

Production of chloroform arrhythmias. Male Sprague-Dawley rats, 100 g, were deprived of food overnight before the experiment but had free access to water. According to a small modification of the method of Erker & Baker (1980), the animals were given intramuscularly 20 mg kg^{-1} aminophylline, and 30 min later placed for 50 s in a 4 L covered beaker containing 200 mL of chloroform. Although most of the chloroform was absorbed by gauze pads on the bottom of the beaker, excess liquid chloroform was always present to maintain a fairly constant vapour pressure. At respiratory arrest, the rats were removed from the beaker, their abdomens and thoraxes were opened without touching the hearts, and the cardiac ventricles were visually examined. The heart was classified as being in a state of spontaneous ventricular arrhythmia if the arrhythmia lasted at least 5 s after the ventricles were exposed for visual examination. If the ventricles were contracting in a coordinated manner they were stimulated by quick pinches with a metal forceps. Drug solutions were given orally in a volume of 2 mL kg^{-1} for 20 s, 30 min before exposure to chloroform.

Pharmacokinetics. Male Sprague-Dawley rats, 340 to 380 g, received digoxin (1 mg kg^{-1}) orally, once a day (between 0900 and 1000h) for 7 days. Just before dosing, blood samples (0·15 mL) were withdrawn from the tail vein, using heparinized syringes, for digoxin determination, and centrifuged at 3000 rev min^{-1} for 10 min with a Hitachi Refrigerated Centrifuge (05 PR-22). Plasma was separated, transferred to test tubes and frozen at −20 °C until assayed (within 2 days). 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 6 groups (n = 7). Group I was treated with digoxin (1 mg kg^{-1}) alone, Group II with digoxin and quinidine (25 mg kg^{-1}), Group III with digoxin and quinidine (50 mg kg^{-1}), Group IV with digoxin and AN-132 (50 mg kg^{-1}), Group V with digoxin and AN-132 (100 mg kg^{-1}), and Group VI with digoxin and AN-132 (200 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, and stored as previously. The drugs were suspended in 3% gum arabic solution, and given by gavage in a volume of 1·0 mL. Plasma digoxin concentrations were measured in duplicate using a radioimmunoassay kit (digoxin ^{125}I kit, Dainabot, Japan). Radioactivity was determined by means of an Aloka γ-counter (model 600). Plasma samples were diluted 10 times with 0·9% NaCl (saline), then 0·1 mL of each sample was used for assay. The area under the plasma concentration time curve

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(AUC_{0-24}) was determined planimetrically. AN=132 and quinidine at 10 mg L^{-1} in saline did not interfere.

The drugs used were digoxin standard (distributed by National Institute of Hygiene, Japan), quinidine (Wako Junyaku) and AN-132, 3-(diisopropylaminoethylamino)-2′,6′-dimethylpropionanilide diphosphate (mol. wt 515·5, $C_{19}H_{33}N_3O{\cdot}2H_3PO_4$, synthesized in our Research Laboratories). Values in the text are represented as means ± s.e. Significant differences between mean values were estimated by using Student's *t*-test; *P* values <0·05 were considered statistically significant.

Results and discussion

The antiarrhythmic effect of AN-132 was evaluated against chloroform-induced arrhythmias in rats. When AN-132 (0·01–3 mg kg^{-1}) was administered orally in increases with a factor of 3, the agent significantly inhibited the incidence of cardiac arrhythmias in a dose-dependent manner. The arrhythmic state occurred in approximately 90% of the hearts of rats not treated with AN-132. However, in the animals treated with AN-132, no arrhythmias took place in the following ratio: 28·6% at 0·01 mg kg^{-1},. 57·1% at 0·1 mg kg^{-1}, 85·7% at 1 mg kg^{-1}, and 100% at 3 mg kg^{-1}. The ED50 (the dose necessary to protect by 50% the occurrence of cardiac arrhythmias) of AN-132 was approximately 0·066 mg kg^{-1} (95% confidence limits, 0·023–0·187 mg kg^{-1}, each n = 7).

The effects of AN-132, compared with quinidine, on plasma digoxin concentrations, were examined in rats. A single 1 mg kg^{-1} dose of digoxin was given orally every 24 h for 7 consecutive days. Venous blood samples were drawn just before each daily dose. Based on all 42 animals measured predose steady-state plasma digoxin concentrations were as follows: 0·76 ± 0·03 ng mL^{-1} before the treatment with digoxin; 1·90 ± 0·08 ng mL^{-1} on day 2; and 1·98 ± 0·14 ng mL^{-1} on day 8. No significant difference was observed between the corresponding values on day 2 and day 8. Thus, in agreement with the previous result (Hinohara et al 1987), predose plasma digoxin levels virtually remained unchanged during 7 consecutive oral digoxin dosing. Fig. 1 represents the plasma digoxin concentration curves for the groups treated orally with digoxin (1 mg kg^{-1}) alone, and together with quinidine (25 or 50 mg kg^{-1}). The plasma concentrations of digoxin attained a peak (C_{max}) approximately 2–4 h after dosing, and decreased gradually, approaching predose values 24 h after dosing. The AUC and C_{max} of digoxin were significantly greater when digoxin was given with quinidine than alone. On the other hand, the co-administration of AN-132, unlike quinidine had no influence on plasma digoxin concentrations. Summarized data are shown in Fig. 1 and Table 1.

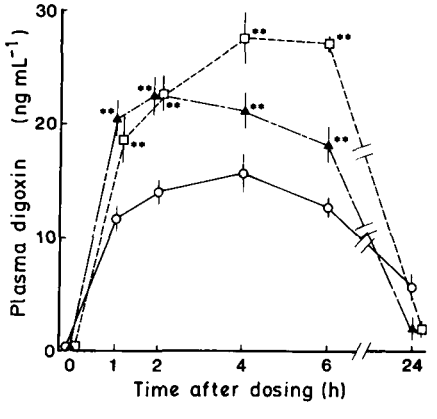

FIG. 1. Plasma digoxin concentrations in chronic digoxin dose study in rats. The vertical bars represent mean ± s.e. of 7 observations on 7 preparations. $^{**}P < 0{\cdot}01$ vs the corresponding value from the group treated with digoxin alone. Key: ○ digoxin 1 mg kg^{-1} alone; ▲ digoxin 1 mg kg^{-1} + quinidine 25 mg kg^{-1}; □ digoxin 1 mg kg^{-1} + quinidine 50 mg kg^{-1}.

Table 1. Effects of AN-132 and quinidine on digoxin kinetics in rats.

	C_{max} (ng mL^{-1})	AUC_{0-24} (ng mL^{-1} h)
Digoxin 1 mg kg^{-1} alone	16·1 ± 1·4	243·0 ± 18·8
Digoxin with AN-132		
50 mg kg^{-1}	18·4 ± 0·6	204·5 ± 16·2
100 mg kg^{-1}	19·6 ± 1·1	240·3 ± 19·2
200 mg kg^{-1}	18·6 ± 0·5	257·4 ± 10·0
Digoxin with quinidine		
25 mg kg^{-1}	23·2 ± 1·4*	299·4 ± 24·5*
50 mg kg^{-1}	29·6 ± 1·0**	396·9 ± 10·0**

Values represented are means ± s.e. (n = 7).
$^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ compared with digoxin alone.

Thus, the present experiment in rats revealed that AN-132 did not affect plasma digoxin concentrations, while quinidine significantly enhanced them. Our results with quinidine are in agreement with clinical findings (Ejvinson 1978; Leahey et al 1978; Chen & Friedman 1980). Baker & Erker (1980) reported that in chloroform-induced arrhythmic preparations of rats, the ED50 of quinidine administered orally was 15 mg kg^{-1}. On the other hand, the ED50 of AN-132 was approximately 0·066 mg kg^{-1} in the present experiment. When compared on the ED50 values, the antiarrhythmic effect of AN-132 on chloroform-induced arrhythmias was approximately 227 times greater than that of quinidine. Taking these together, it is concluded that the lack of any effect of AN-132 on digoxin plasma levels in the present study is not ascribable to insufficient dosing.

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