Investigation of Digoxin, Quinidine, and Disopyramide Interactions in Rats Utilizing Parotid Saliva, Blood, and Other Tissues

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Abstract □ Blood, parotid saliva, heart, liver, and kidney concentrations of digoxin and quinidine were determined in rats chronically treated with digoxin and in nontreated (control) rats after the administration of quinidine (20 mg/kg ip) and disopyramide (10 mg/kg ip). The results indicated that digoxin concentrations increased significantly and proportionally in parotid saliva and plasma after quinidine, but did not increase after disopyramide. With the exception of the liver, which showed an increase in digoxin concentrations, tissue concentrations of digoxin did not differ from control animals. In rats pretreated chronically with digoxin, quinidine concentrations in plasma, parotid saliva, or heart tissue did not differ significantly from control animals, but were significantly lower than controls in liver and kidney tissues. The results presented here lend additional support to the hypothesis that the increase in digoxin plasma concentration following quinidine administration is primarily due to interference with renal excretion and displacement of digoxin by quinidine binding sites. Furthermore, it was demonstrated that disopyramide has little or no effect on plasma digoxin levels in rats.

Keyphrases □ Digoxin—interaction with quinidine and disopyramide in parotid saliva, blood, and other tissues □ Quinidine—interaction with digoxin and disopyramide in parotid saliva, blood, and other tissues □ Disopyramide—interaction with digoxin and quinidine in parotid saliva, blood, and other tissues

Considerable attention has been focused on the mechanism of the drug interaction between digoxin and quinidine (1–7). Several mechanisms have been proposed, but because of the limited flexibility of the human model, they cannot be supported by hard experimental evidence. Relatively few animal studies have been employed to evaluate the digoxin–quinidine interaction.

The present investigation evaluated the interaction between digoxin and quinidine and digoxin and disopyramide in rats. Blood, parotid saliva, and tissue concentrations of digoxin were determined in rats pretreated chronically with digoxin and later administered single doses of quinidine or disopyramide. In addition, blood, parotid saliva, and tissue concentrations of quinidine were determined in rats chronically pretreated with digoxin and later administered a single dose of quinidine.

EXPERIMENTAL

Reagents and Materials—all reagents were analytical grade, and all aqueous solutions were made with glass-distilled water. Digoxin[1] and pentobarbital sodium[2] were obtained in the injectable form. Quinidine[3] and disopyramide[4] were obtained in powdered form and appropriate solutions were made. Cannulas for the collection of whole blood and parotid saliva were fabricated from polyethylene 50 and 10 tubing[5]. Samples collected were assayed for digoxin and quinidine content using enzyme immunoassay kits[6] in accordance with the manufacturer's instructions.

Experiment 1—*Comparison of Digoxin Concentrations in Parotid Saliva, Plasma, Heart, Liver, and Kidney Tissues of Rats Administered Quinidine, Disopyramide, and Saline*—Male Wistar rats, 150–170 g, were housed in individual cages and provided with a standard diet of laboratory chow[7] and distilled water containing digoxin (2.5 μg/ml) *ad libitum*. The volume of water consumed and weight gained were recorded daily for each animal. After 8 days of drinking digoxin-containing water, a total of 37 animals were divided into three experimental groups, A, B, and C (Scheme I). These groups were designated to receive saline, quinidine (20 mg/kg ip) and disopyramide (10 mg/kg ip), respectively.

All animals were prepared surgically for collection of parotid saliva and whole blood using pentobarbital (50 mg/kg ip) anesthesia according to an earlier method (8). A tracheotomy was performed and with the aid of a dissecting microscope, the right brachial artery, right femoral artery, and both parotid ducts were surgically exposed for cannulation. The brachial and femoral arteries were cannulated with polyethylene 50 tubing and the parotid ducts were cannulated with tapered polyethylene 10 tubing. The femoral artery was used to obtain blood samples. The brachial artery was used for the constant infusion of the secretagogue, pilocarpine (0.25 mg/ml) at a rate of 0.21 ml/min. The parotid cannulas were directed into a disposable glass culture tube for the collection of saliva.

Following surgery, Group A animals received an intraperitoneal challenge injection of saline, Group B, an intraperitoneal challenge injection of quinidine, and Group C received an intraperitoneal challenge injection of disopyramide. Thirty minutes after the intraperitoneal challenge, the collection of parotid saliva was started and continued over

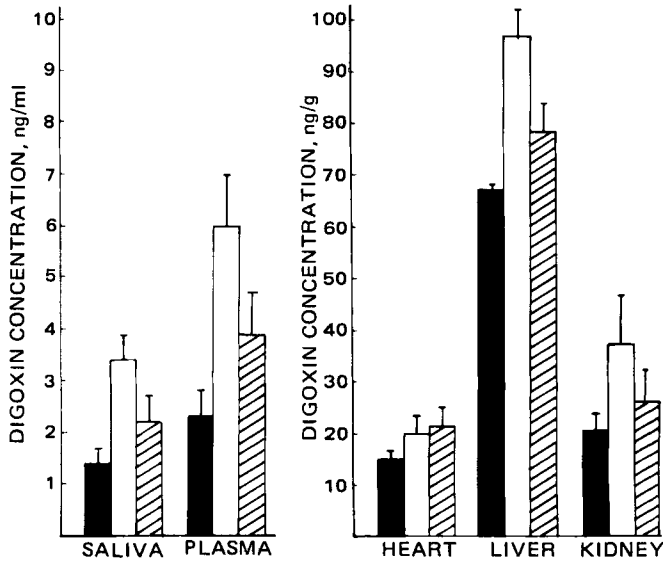

Figure 1—*Comparison of digoxin concentrations in parotid saliva, plasma, heart, liver, and kidney tissues of digoxin chronically treated rats after saline (■, Group A), quinidine (□, Group B), and disopyramide (▨, Group C).*

[1] Lanoxin, Burroughs Wellcome Co., Research Triangle Park, N.C.
[2] Nembutal, Abbott Laboratories, North Chicago, Ill.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Searle, Chicago, Ill.
[5] Fisher Scientific, King of Prussia, Pa.
[6] EMIT (Enzyme-multiplied immunoassay technique), Syva Co., Palo Alto, Calif.
[7] Ralston Purina, St. Louis, Mo.

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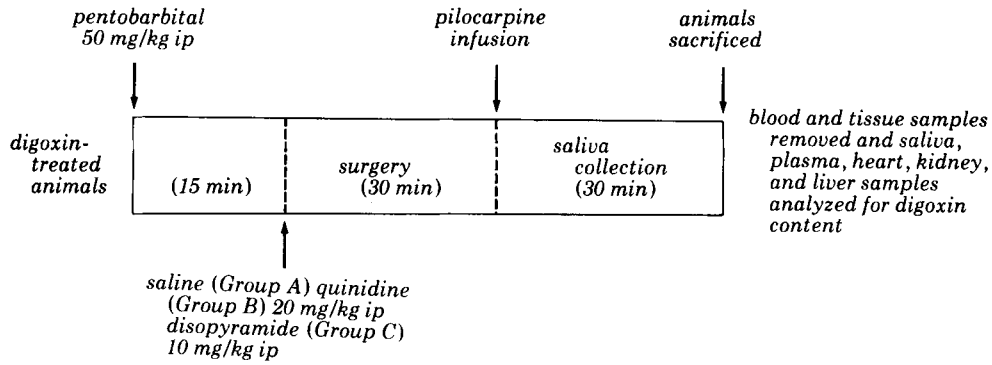


Scheme I—Diagram of experiment 1

the next 30 min. Subsequently, 5 ml of whole blood was removed from the femoral artery and placed in a heparinized tube. The animal was then sacrificed and the heart, liver, and kidneys were removed; whole blood was centrifuged and the plasma was removed. All samples were frozen until the assay. The sequence of these events is presented in Scheme I.

Heart, liver, and kidney samples were weighed and 0.75–1.00 g of tissue was homogenized in 5 ml of 0.9% sodium chloride and mixed with 10 ml of methylene chloride (9). After centrifugation, 5 ml of the organic layer was decanted and evaporated. The residue was dissolved in 5 or 10 ml of distilled water (10). All samples were assayed for digoxin content by enzyme immunoassay.

Experiment 2—*Comparison of Quinidine Concentration in Parotid Saliva, Plasma, Heart, Liver, and Kidney Tissues of Rats Pretreated with Digoxin and Saline*—Male Wistar rats, 150–170 g, were housed in individual cages and given a standard diet of laboratory chow *ad libitum*. Control animals (Group A) were provided tap water *ad libitum*, while experimental animals (Group B) were provided tap water containing digoxin (2.5 μg/ml) *ad libitum* (Scheme II). Water consumption and weight gain were recorded daily for both groups. After 8 days, both groups of rats underwent surgical preparation as described for Experiment 1.

Quinidine (20 mg/kg ip) was administered to Group A and Group B animals. Parotid saliva was collected over 30 min after which each animal was sacrificed and blood, liver, heart, and kidney samples were removed. Samples were handled in a manner similar to that described in Experiment 1. However, in this experiment, samples were assayed for quinidine concentration using enzyme immunoassay.

RESULTS

As described, the rats given digoxin-containing water (2.5 μg/ml) for 8 days were divided into three groups based on the administration of a second drug on the 8th day (Table I): Group A (control animals), which received only digoxin, Group B (quinidine animals), and Group C (disopyramide animals).

Daily digoxin consumption and body weight in these three groups were compared. Using the Student *t* test, no significant differences in the daily digoxin consumption or body weights could be found among any of the groups ($p > 0.05$).

Comparison of Digoxin Concentrations in Parotid Saliva, Plasma, and Heart, Liver, and Kidney Tissues of Rats Administered Quinidine, Disopyramide, and Saline—Digoxin concentrations in the parotid saliva, plasma, liver, kidney, and heart tissues in rats consuming digoxin for 8 days were measured in each of three experimental groups with the following results (Fig. 1).

The digoxin content of saliva and plasma collected from Group A (control) animals was 1.4 ± 0.3 and 2.3 ± 0.5 ng/ml, respectively. Heart, liver, and kidney tissue displayed digoxin concentrations of 14.8 ± 1.7, 67.0 ± 1.0, and 19.7 ± 3.8 ng/g, respectively.

Group B (quinidine treated) rats displayed a digoxin concentration in parotid saliva of 3.4 ± 0.5 ng/ml and a plasma concentration of 6.0 ± 1.0 ng/ml. When the hearts, livers, and kidneys of these animals were homogenized and analyzed they were found to contain 20.0 ± 4.0, 96.4 ± 5.7, and 37.1 ± 9.4 ng/g of digoxin, respectively.

Group C (disopyramide treated) rats displayed saliva and plasma digoxin levels of 2.2 ± 0.5 and 3.9 ± 0.8 ng/ml, respectively. Analysis of their heart, liver, and kidney tissue revealed digoxin levels of 21.7 ± 3.5, 78.4 ± 7.1, and 26.0 ± 6.2 ng/mg, respectively.

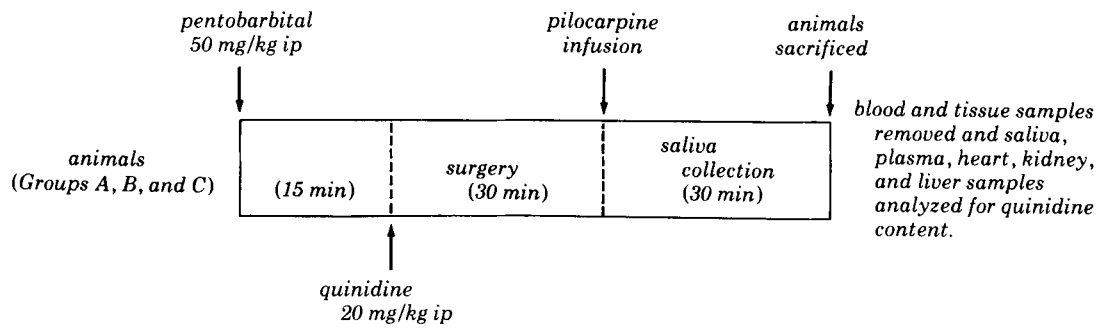
When evaluated using the Student *t* test, the digoxin content of parotid saliva, plasma, and liver was significantly higher in Group B rats than in control animals $p < 0.01$, $p < 0.005$, and $p < 0.005$, respectively. No significant differences in digoxin concentration could be found between control rats and Group C animals for any tissue studied ($p > 0.05$).

The mean ratios (±*SE*) between parotid saliva digoxin concentrations and those found in plasma were 0.60 ± 0.10, 0.69 ± 0.11, and 0.68 ± 1.20 for the Group A, B, and C rats, respectively. For Groups A, B, and C, the mean ratios (±*SE*) between the digoxin concentrations found in liver and in plasma were calculated to be 33.8 ± 8.7, 29.0 ± 8.60, and 35.2 ± 10.40; for kidney and plasma, 8.0 ± 1.60, 8.9 ± 2.10, and 8.7 ± 1.70; and finally for heart and plasma, 9.3 ± 2.80, 5.3 ± 1.60, and 7.3 ± 1.80.

Comparison of Quinidine Concentration in Parotid Saliva, Plasma, Heart, Liver, and Kidney Tissues of Rats Pretreated with Digoxin and Saline—Control animals displayed quinidine concentrations (Fig. 2) of 0.3 ± 0.1 ng/ml (parotid saliva) 1.1 ± 0.8 ng/ml (plasma), 1.0 ± 0.2 ng/g (heart), 0.6 ± 0.2 ng/g (liver), and 1.3 ± 0.4 ng/g (kidney). Parotid saliva and plasma were analyzed in Group B rats and found to contain quinidine in concentrations of 0.4 ± 0.04 and 0.9 ± 0.1 ng/ml, respectively. Homogenates of heart, liver, and kidney tissues taken from these animals contained 0.6 ± 0.2, 0.3 ± 0.05, and 0.5 ± 0.1 ng of quinidine/g, respectively. When these quinidine concentrations were compared with those obtained in control animals using the Student *t* test, the following results were noted. No significant variation between the groups was found for quinidine concentrations detected in parotid saliva ($p > 0.05$), plasma ($p > 0.05$), or heart tissue ($p > 0.05$). The quinidine concentrations in the liver and kidney tissue of Group A animals were significantly higher than those of Group B animals ($p < 0.05$).

DISCUSSION

Some controversy exists concerning the mechanism of the interaction reported to occur between quinidine and digoxin. The most frequently



Scheme II—Diagram of experiment 2

Table I—Comparison of Weights and Digoxin Consumption by Three Groups of Rats over 8 Days

Group[a]	n	Weight[b], Mean, g	Digoxin[c] Consumed in 24 hr, ng
A	10	255 ± 4.4	103.0 ± 6.8
B	10	259 ± 4.6	104.0 ± 5.0
C	10	253 ± 7.9	122.2 ± 8.0

[a] Group A animals received digoxin only; Group B received digoxin and quinidine; and Group C received digoxin and disopyramide. [b] Mean (±*SE*) of weights of rats fed over 8 days. [c] Mean (±*SE*) of digoxin consumed per 24 hr from drinking water containing 2.5 μg of digoxin/ml over 8 days.

proposed mechanisms include the displacement of digoxin by quinidine from tissue binding sites (2, 5) and the interference by quinidine with renal digoxin excretion (11).

During the 8 days of digoxin consumption, animals in all groups consumed approximately the same quantity of digoxin. In addition, the mean weights after pretreatment with digoxin did not differ from group to group. The results indicated that during these 8 days, all the animals reacted similarly, so there was little or no intra-individual variation at the time of the experiment.

Results of the present investigation support two possible mechanisms for this interaction: (*a*) quinidine interferes with the renal excretion of digoxin although renal clearance of digoxin has not been measured, and (*b*) quinidine interferes with digoxin tissue binding sites.

Following quinidine administration, digoxin concentrations increased significantly in plasma and parotid saliva compared with control animals. The increases were proportional because the saliva/plasma ratios for control animals and the experimental group did not significantly differ from each other. In liver tissue, digoxin concentrations increased after quinidine administration; however, kidney and heart digoxin concentrations did not significantly differ from those of control animals. The increase in plasma was greater than any of the tissue concentration changes. Subsequently, the liver and cardiac muscle tissue/plasma concentration ratios actually decreased after quinidine administration, whereas the kidney/plasma ratio did not significantly change. These results were similar to other findings (12) where the corresponding digoxin tissue/serum ratios decreased in dogs after quinidine.

Assuming the increase in plasma digoxin concentrations involves an interference with renal digoxin excretion by quinidine, proportional increases in the plasma and parotid saliva digoxin concentrations and possible increases in tissue concentrations would be expected. This hypothesis is supported by previous investigations (13–15) that demonstrated that increasing the plasma concentrations of various drugs causes proportional increases in the free nonprotein-bound plasma drug levels as well as those in the parotid saliva. However, if the increases in plasma digoxin concentration after quinidine administration were due solely to a decrease in plasma protein or tissue digoxin binding, one would anticipate that: (*a*) parotid saliva concentrations would increase to a greater proportion than plasma with a subsequent increase in the saliva/plasma ratio, and/or (*b*) tissue digoxin concentrations would decrease significantly due to the displacement of digoxin by quinidine.

As previously discussed, saliva/plasma ratios did not change from control values, but the liver and cardiac digoxin tissue concentrations showed a tendency to decrease when compared with the plasma concentrations. It is for these reasons that the present results support more than one mechanism for this interaction which may involve tissue binding sites and renal clearance.

Other investigators (1, 16) reported that various antiarrhythmic agents (including disopyramide) had little or no effect on digoxin blood levels. Results also indicate that there are no significant differences between digoxin concentrations in plasma, parotid saliva, and heart, liver, and kidney tissues determined before and after disopyramide administration.

In the second experiment, quinidine concentrations in blood and parotid saliva showed no significant differences between control and digoxin-treated animals. In addition, quinidine concentrations tended

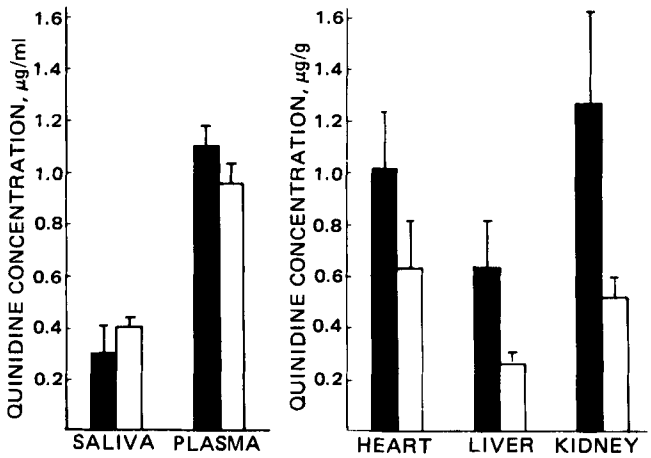


Figure 2—*Comparison of quinidine concentrations in parotid saliva, plasma, heart, liver, and kidney tissues in control rats (■, Group A) and in digoxin chronically treated rats (□, Group B).*

to decrease in liver and kidney tissue. As previously discussed, if the interaction was mainly due to the ability of quinidine to displace digoxin from plasma proteins binding sites, the free plasma concentrations of quinidine or parotid saliva quinidine would be expected to decrease. However, if quinidine significantly occupied tissue binding sites previously occupied by digoxin then the quinidine tissue concentrations would tend to increase over control. In neither instance did the earlier results correlate with an interference of plasma protein or tissue binding. However, if the interaction of quinidine–digoxin was controlled only by a renal mechanism, then quinidine concentrations in plasma and/or parotid saliva would probably not differ from those of controls as observed. However, while one would expect that quinidine concentrations in kidney would increase under this mechanism, the results presented here actually show the opposite effect, suggesting the possibility that more than one mechanism is involved. Studies are in progress to investigate these findings further.

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