Myocardial Monovalent Cation Transport during the Quinidine-Digoxin Interaction in Dogs

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SUMMARY. To study the relationship of the serum digoxin concentration to the digoxin effect on monovalent cation transport during the quinidine-digoxin interaction, we used radiolabeled rubidium to measure monovalent cation active transport in myocardial biopsy samples from dogs. In a preliminary study, we showed that quinidine did not affect rubidium uptake by myocardial samples from intact dogs. Then, we studied four groups, each consisting of 13 dogs, which received either saline, low dose digoxin, high dose digoxin, or low dose digoxin plus quinidine treatment. In these groups of dogs, the following steady state serum digoxin concentrations were achieved: saline-treated, 0 ng/ml; low dose digoxin, 1.2 ± 0.2 ng/ml (mean ± SD); high dose digoxin, 2.4 ± 0.4 ng/ml; and low-dose digoxin plus quinidine treated, 2.3 ± 1.1 ng/ml. Compared to control values, rubidium uptake was decreased by 17% in dogs treated with low dose digoxin (P < 0.05) and by 38% in dogs treated with high dose digoxin (P < 0.01 vs. saline-treated, P < 0.01 vs. low dose digoxin). Although low dose digoxin plus quinidine-treated dogs had the same mean serum digoxin concentration as the high dose digoxin-treated dogs, rubidium uptake in low dose digoxin plus quinidine-treated dogs was decreased by only 17% compared to control (P < 0.05 vs. saline-treated, P < 0.01 vs. high dose digoxin). During the quinidine-digoxin interaction in the intact dog, the reduction in myocardial rubidium uptake is less than expected from the increase in serum digoxin concentration. (Circ Res 54: 453-460, 1984)

IT is not known whether or not the effect of digoxin increases when the serum digoxin concentration (SDC) rises during the quinidine-digoxin interaction (Bigger, 1982; Bussey, 1982; Doherty, 1982). Some authors (Leahey et al., 1978; Manyari et al., 1981; Reid and Meek, 1979; Pieroni and Marshall, 1981) report evidence of digoxin toxicity during the quinidine-digoxin interaction. Others (Schenk-Gustaffson et al., 1981; Steiness et al., 1980; Hirsch et al., 1980) have failed to find evidence of toxicity, and report that the inotropic effect of digoxin does not increase when the SDC rises during quinidine treatment. It is difficult to delineate the relationship between digoxin dose (or SDC) and myocardial digoxin effect in the presence of quinidine, because quinidine can produce toxic symptoms similar to those of digoxin. This difficulty has contributed to the variability of the results obtained in clinical studies. In order to provide a better understanding of the significance of the quinidine-digoxin interaction, it is important to clarify the relationship between the SDC and the toxic and therapeutic effects of digoxin during quinidine administration.

Accordingly, we studied the relationship between SDC and digoxin effect during the quinidine-digoxin interaction, using inhibition of myocardial monovalent cation transport as the measure of myocardial digoxin effect. Measurement of myocardial monovalent cation transport by Rb⁺ uptake has two advantages over methods used previously to measure digoxin's effect, for the study of the quinidine-digoxin interaction. First, the magnitude of inhibition of Rb⁺ uptake is proportional to the digoxin dose (Hougen et al., 1979). Second, therapeutic concentrations of quinidine have no measurable effect on Rb⁺ uptake in the models studied to date (Ball et al., 1981; Kim et al., 1981a).

Our working hypothesis was that the quinidine-induced increase in SDC is accompanied by a corresponding decrease in myocardial Rb⁺ uptake. We designed study I, using fixed drug doses in mg/kg body weight, to test this hypothesis. The results of study I were inconclusive, because the interindividual variability in SDC resulted in overlapping of SDC between the group of dogs given digoxin and the group given digoxin and quinidine. Therefore, in study II, we adjusted the doses of digoxin and quinidine to reduce the variability in SDC within the study groups.

Methods

Protocols

The treatment regimens for dogs in studies I and II are shown in Table 1.
TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Protocols for Studies I and II</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>Fixed drug doses</td>
</tr>
<tr>
<td>IB</td>
<td>200 mg tpd</td>
</tr>
<tr>
<td>IC</td>
<td>100 mg tpd</td>
</tr>
<tr>
<td>ID</td>
<td>200 mg tpd</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>Saline</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>Quinidine sulfate po</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>400 mg tpd</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>Digoxin iv 40 µg/kg</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>400 mg tpd</td>
</tr>
</tbody>
</table>

First treatment period (day 1-7)

Saline Quinidine sulfate po 400 mg tpd Digoxin iv 40 µg/kg and quinidine sulfate po 400 mg tpd

Second treatment period (day 5–15)

Saline Quinidine sulfate po 400 mg tpd Digoxin iv 20 µg/kg and quinidine sulfate po 400 mg tpd

Dose-ranging

Saline Digoxin iv (5-15 µg/kg) to steady state SDC of 1-1.5 ng/ml Digoxin iv (5-15 µg/kg) to steady state SDC of 1-1.5 ng/ml

Group IIA (n = 13) Group IIB (n = 13) Group IIC (n = 13) Group IID (n = 13)

[125I]-radioimmunoassay was used to measure serum digoxin concentration in dogs in the fixed drug dose studies (Taubert and Shapiro, 1975). Between the two studies, [3H]digoxin radioimmunoassay was introduced into the laboratory and was used to measure SDC of 22 of the dogs in the dose-ranging study (Smith et al., 1969). Preparation and characterization of the antibody used in the [3H]digoxin radioimmunoassay has been described by Smith et al. (1970). Digoxin used as a tracer in [3H]digoxin radioimmunoassay was specifically labeled at the 12-alpha site (New England Nuclear). Seventeen of the dogs in the dose-ranging study received digoxin that was specifically labeled with tritium with appropriate corrections for quenching and chemiluminescence (Warner et al., 1983).

Quinidine and quinidine metabolites were extracted from serum by the method of Cramer and Isaksson (1963).
and measured by HPLC with fluorescence detection. Quinidine was the internal standard in this assay.

**Measurement of Rb* Uptake**

Dogs were anesthetized with 30 mg/kg intravenous pentobarbital, intubated with a cuffed endotracheal tube, and ventilated with a Harvard positive pressure respirator. Arterial blood gases were measured periodically, and ventilation was adjusted to maintain pH 7.30–7.50 and PO₂ above 70 mm Hg. Leads I and II of the electrocardiogram were continuously monitored, as was the arterial blood pressure, through a femoral artery catheter.

A thoracotomy was performed through the left 5th intercostal space, the heart exposed, and the pericardium opened. Blood samples were obtained from the femoral artery for measurement of arterial pH, PCO₂, P O₂, serum Na⁺, K⁺, digoxin, and quinidine concentrations. Five transmural biopsies of the left ventricular myocardium, 4 mm in diameter and weighing approximately 50 mg each, were obtained from the beating heart with a hand-held drill. Biopsy samples were immediately placed in 15–20 ml of buffer containing 4.0 mM KCl, 120 mM NaCl, 24 mM NaHCO₃, 2.0 mM MgCl₂, 2.5 mM CaCl₂, 5.6 mM glucose, and 1.1 mM NaH₂PO₄. All buffer used in this study was equilibrated with 95% O₂ and 5% CO₂ and had a pH after equilibration of 7.20 ± 0.10. Myocardial samples were sliced into 20–30 1 X 2 X 5-mm samples while in buffer. The samples were placed in flasks containing 15–20 ml of buffer for 5 minutes. Tissue samples were then transferred to flasks containing 20 ml of buffer with 10⁻³ M ouabain. Passive Rb⁺ uptake was calculated from samples incubated in medium without ouabain. After incubation, the tissue samples were rinsed 3 times in 300–500 ml of buffer and blotted, and weighed. Total Rb⁺ uptake was determined by subtracting passive Rb⁺ uptake from total Rb⁺ uptake.

**Statistical Analysis**

Student's t-test was used to compare the mean SDC of the two groups of dogs that received digoxin (IC, ID) and the mean serum quinidine concentrations of the two groups of dogs that received quinidine (IB, ID) in the fixed drug dose study. A paired t-test was used to compare the SDC of the dogs in group IID before quinidine with the SDC after quinidine treatment.

One-way analysis of variance with a priori contrasts was used to compare the SDC in the three groups of dogs that received digoxin in the dose-ranging study. In both the fixed dose and dose-ranging studies, analysis of variance with contrasts was used to evaluate differences in Rb⁺ uptake between the treatment groups. Analysis of variance was used to look for differences in serum Na⁺, K⁺ and arterial blood gases among the treatment groups in both studies.

Regression analysis was used to quantify the relationships between: (1) serum quinidine and serum digoxin concentrations, (2) serum digoxin concentration and Rb⁺ uptake, (3) Rb⁺ uptake and serum K⁺, (4) Rb⁺ uptake and serum Na⁺, (5) Rb⁺ uptake and arterial pH, (6) Rb⁺ uptake and arterial P CO₂, and (7) Rb⁺ uptake and arterial P O₂.

**Results**

**Fixed Drug Dose Study (I)**

The mean serum digoxin concentration of the dogs that received digoxin alone (group IC) was 2.7 ± 0.8 ng/ml (mean ± sd), which was different at the P < 0.05 level from the mean SDC of 3.3 ± 0.7 ng/ml of the dogs that received digoxin and quinidine (group ID). There was no significant difference between the mean serum quinidine concentration of 5.1 ± 2.4 μg/ml of the dogs that received quinidine alone (group IB) and the mean serum quinidine concentration of 3.9 ± 2.3 μg/ml of the dogs that received digoxin and quinidine (group ID).

Monovalent cation transport as measured by active Rb⁺ uptake in the four treatment groups in the fixed drug dose study is shown in Table 2. Quinidine had no effect on Rb⁺ uptake, i.e., Rb⁺ uptake was not different in groups IA and IB. Active Rb⁺ uptake was significantly lower in both groups that received digoxin compared to those that did not (groups IA and IB) (P < 0.02). The Rb⁺ uptake of the group that received only digoxin (group IC) was not different from that in the group that received digoxin and quinidine (group ID).

**Dose-Ranging Study (II)**

**Time course of the Quinidine-Digoxin Interaction**

The mean serum quinidine concentration in group IID at the time of measurement of myocardial monovalent cation transport was 4.1 ± 2.2 μg/ml (range 2.2–10.4 μg/ml). Figure 1 shows the mean serum quinidine concentration of the dogs on digoxin alone (group IIB) and the mean serum digoxin concentration in both studies. The average SDC of the 13 dogs continued on digoxin alone (group IIB) is also shown. In most dogs, the SDC began to rise within 24 hours of quinidine administration. After 72 hours of quinidine, the difference between the SDC of dogs in group IID and the SDC of dogs in group IIB was significant (P < 0.05). Quinidine was given for a total of 4–8 days, at which time the mean SDC of the dogs in group IIB had increased by 98% from the pre-quinidine SDC. Dogs continued on digoxin without quinidine (group IIB) had a SDC of 1.2 ± 0.2 ng/ml at the onset of the second treatment period (see protocol in Table 1) and a SDC of 1.2 ± 0.2 ng/ml after an additional 4–8 days of digoxin dosing.

Figure 2 shows the SDC at the time of measurement of myocardial monovalent cation transport for...
TABLE 2

Fixed Dose Study of Quinidine-Digoxin Interaction (Study I)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Treatment</th>
<th>Serum digoxin concentration (ng/ml)</th>
<th>Serum quinidine concentration (μg/ml)</th>
<th>Rubidium uptake (nmol/mg per 30 min)</th>
<th>% Change in rubidium uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9</td>
<td>Saline</td>
<td>2.7 ± 0.8</td>
<td>5 ± 2.4</td>
<td>0.149 ± 0.037</td>
<td>+5%</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>Quinidine</td>
<td>2.3 ± 0.2</td>
<td>5.1 ± 2.4</td>
<td>0.156 ± 0.052</td>
<td>-28%</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>Digoxin</td>
<td>3.3 ± 0.7</td>
<td>3.9 ± 2.3</td>
<td>0.107 ± 0.025*</td>
<td>-23%</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>Digoxin; quinidine</td>
<td>3.3 ± 0.7</td>
<td>3.9 ± 2.3</td>
<td>0.115 ± 0.051*</td>
<td>-23%</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.

*P < 0.05 compared to control rubidium values.

Each dog in the three experimental groups that received digoxin (groups IIB, IIC, IID) as well as the pre-quinidine SDC of the dogs that received quinidine and digoxin (group IID). One-way analysis of variance with a priori contrasts was used to evaluate between-group differences in SDC at the time of measurement of monovalent cation transport. The null hypothesis that the SDC of the three groups are the same was rejected (P < 0.01). At the time of measurement of monovalent cation transport, dogs in group IIB (low dose digoxin) had a SDC of 1.2 ± 0.2 ng/ml, different both from the SDC of 2.4 ± 0.4 of the dogs in group IIC (high dose digoxin) (P < 0.01) and from the SDC of 2.3 ± 1.1 of the dogs in group IID (low dose digoxin plus quinidine) (P < 0.01). The mean SDC of group IIC (high dose digoxin) was not different from the mean SDC in group IID (low dose digoxin plus quinidine).

Myocardial Monovalent Cation Transport

Active Rb⁺ uptake in the four experimental groups in study II is presented in Table 3. Dogs in Group...
TABLE 3
Dose-Ranging Study of Quinidine-Digoxin Interaction (Study II)

<table>
<thead>
<tr>
<th>Group II</th>
<th>n</th>
<th>Treatment</th>
<th>Serum digoxin concentration (ng/ml)</th>
<th>Serum quinidine concentration (μg/ml)</th>
<th>Rubidium uptake (nmol/mg per 30 min)</th>
<th>% Change in rubidium uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13</td>
<td>Saline</td>
<td></td>
<td></td>
<td>0.205 ± 0.040</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>13</td>
<td>Digoxin (low dose)</td>
<td>1.2 ± 0.2</td>
<td></td>
<td>0.171 ± 0.042*</td>
<td>−17%</td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>Digoxin (high dose)</td>
<td>2.4 ± 0.4</td>
<td></td>
<td>0.128 ± 0.032†</td>
<td>−38%</td>
</tr>
<tr>
<td>D</td>
<td>13</td>
<td>Digoxin; quinidine (low dose)</td>
<td>2.3 ± 1.1</td>
<td>4.1 ± 2.2</td>
<td>0.170 ± 0.044*</td>
<td>−17%</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
* P < 0.05 compared to control rubidium values.
† P < 0.001 compared to control rubidium values.

IIIB (SDC = 1.2 ± 0.2 ng/ml) had a reduction of 17% Rb+ uptake compared to the control group (P < 0.05). The Rb+ uptake of dogs in group IIIC (SDC = 2.4 ± 0.4 ng/ml) was reduced by 38% compared to control (IIA) (P < 0.001) and by 25% compared to group IIIB (P < 0.01). The dogs that received low dose digoxin and quinidine (group IID) had a SDC that was almost identical to that in group IIIC (high dose digoxin), but the Rb+ uptake was decreased by only 17% compared to the 38% found in group IIIC (P < 0.01). In fact, the reduction in Rb+ uptake in group IID (low dose digoxin plus quinidine) was identical to that in group IIIB where the SDC was half as great.

Figure 3 shows the regression of myocardial Rb+ uptake on SDC for the 26 dogs (groups IIIB and IIIC) that received digoxin only. The formula for the regression is: Rb+ uptake = 0.203 - 0.03 X SDC. The correlation coefficient is 0.50 and the regression coefficient is different from zero at the P < 0.02 level. The formula for the regression line for group IID is y = 0.222 - 0.02 X SDC. The correlation coefficient for SDC vs. Rb+ uptake for the dogs that received digoxin and quinidine (group IID) was 0.53 (regression coefficient different from zero at P = 0.06). There is a statistically significant difference (P < 0.05) between the two regression lines, indicating that quinidine alters the relationship between SDC and active myocardial Rb+ uptake. During quinidine treatment (group IID), the relationship between SDC and active Rb+ uptake is altered so that, at the same SDC, the reduction in myocardial monovalent cation transport is much less than expected.

Table 4 shows the mean serum Na+, serum K+, and arterial blood gas determinations for the four groups of dogs in study II. There are no differences among the four groups. Within experimental groups, myocardial Rb+ uptake is not related to any of these measurements.

Discussion

Studies from several laboratories have shown that the inotropic effect of digoxin is associated with an inhibition of myocardial Na,K-ATPase, which results in a reduction in monovalent cation transport (Aker et al., 1970; Besch et al., 1970; Langer, 1977;
and the dependent variable, active myocardial Rb⁺ uptake, had decreased our ability to detect differences between the groups. Specifically, we were concerned that the observed increase in SDC caused by quinidine treatment was not sufficient to produce a measurable change in Rb⁺ uptake. We therefore designed the second study to produce larger differences in SDC among the groups of dogs, to permit less between-animal variability in SDC, and to include a critical comparison group, i.e., dogs treated with digoxin alone in doses that produced an average SDC equal to the average SDC of the dogs treated with digoxin and quinidine. We did not include a quinidine control group in study II because we had shown in study I that even high concentrations of quinidine in serum (5.1 ± 2.4 mg/ml) did not affect myocardial Rb⁺ uptake in intact dogs. The average serum quinidine concentration of the dogs given digoxin and quinidine in study II (4.1 ± 2.2 mg/ml) was not significantly different from that of dogs given quinidine alone in study I (5.1 ± 2.4 μg/ml).

There are two previous studies of myocardial monovalent cation transport during the quinidine-cardiac glycoside interaction. Kim et al. (1981a) measured 42K transport in isolated electrically stimulated left atrial muscle from rat hearts incubated with ouabain and quinidine. They found that quinidine did not alter the effect of ouabain on myocardial monovalent cation transport. In separate studies of Langendorff preparations from guinea pigs and rats, they found that quinidine did not alter the relationship between ouabain concentration in the perfusing solution and the inotropic effect of ouabain. One obvious difference between this study and ours was the use of a different cardiac glycoside. In subsequent studies in guinea pigs, Kim and coworkers (1981b) demonstrated that quinidine caused changes in digoxin binding to tissue receptors but did not affect ouabain binding, suggesting that the quinidine-ouabain interaction differs from the quinidine-digoxin interaction in the guinea pig. The study of monovalent cation transport done by Kim et al. (1981a) differs from ours in several other ways. We used dogs, gave the drugs in vivo, measured SDC to confirm the drug interaction, measured monovalent cation transport in left ventricular biopsy samples, and did not electrically stimulate the myocardium during measurement of myocardial monovalent cation transport. Kim et al. (1981a), in contrast, used isolated rat atrial tissue, which was incubated with different concentrations of ouabain and quinidine and electrically stimulated during measurement of myocardial monovalent cation transport.

Horowitz et al. (1982) studied the quinidine-digoxin interaction in cultured chick embryo heart cells. In this system, the presence of quinidine did not appear to alter either contractility or Rb⁺ uptake of cultured cells exposed to digoxin. Horowitz et al.
needed to further elucidate the finding that the effect of digoxin on monovalent cation transport is less than predicted from the SDC. During quinidine treatment, more digoxin may be bound nonspecifically, rather than to active sites in the heart, and/or the digoxin bound to active sites may not produce its usual effect because quinidine interferes with some aspect of the digoxin receptor-effector mechanism.

Extrapolation of the results of this study to the clinical setting must be done with great circumspection. Nevertheless, the finding of this study, that quinidine produces a dissociation of SDC from digoxin's effect on myocardial monovalent cation transport, raises questions about the value of the SDC as a predictor of the effect of digoxin during the quinidine-digoxin interaction. Inhibition of myocardial monovalent cation transport is related to the effect of cardiac glycosides on contractility and ventricular arrhythmias (Hougen et al., 1979). Patients receiving both digoxin and quinidine may therefore have less inotropic effect and also less likelihood of developing AV junctional or ventricular tachyarrhythmia at a given SDC than patients receiving digoxin alone. On the other hand, the effect of digoxin on the sinus node and AV node is largely indirect, mediated through the autonomic nervous system. There is clinical evidence that the relationship between SDC and AV conduction is not altered by quinidine (Leahey et al., 1980). Furthermore, there is no evidence that the relationship between SDC and neurally mediated toxicity, including gastrointestinal symptoms, is altered by quinidine. If digoxin and quinidine are used together, we recommend that the digoxin dosing be based on careful clinical evaluation, as well as SDC measurements, until the relationship between the SDC and the clinical effects of digoxin during the quinidine-digoxin interaction is clarified further.

References


INDEX TERMS: Digoxin • Quinidine • Drug interaction • Monovalent cation transport
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