EFFECTS OF DILTIAZEM ON PLASMA AND TISSUE DIGOXIN LEVELS IN MICE*

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Effects of diltiazem hydrochloride (DTZ) on digoxin (DX) concentrations in plasma and tissues (brain, heart, liver, and kidney) were studied in mice, and the effects were compared with those of quinidine sulfate (QD). When DX (0.2 mg/kg) was co-administered with DTZ (60 mg/kg) orally for 5 d to mice, plasma DX concentrations were increased significantly as with QD (100 mg/kg). Tissue DX concentrations were also increased in brain, heart, and liver. However, the DX tissue/plasma concentration (T/P) ratios for brain, heart, and kidney were rather decreased with DTZ or QD.

The increased plasma and tissue DX concentrations and the decreased T/P ratios with DTZ might be responsible for both the displacement of tissue DX binding and reduced DX elimination as with QD.

Keywords — digoxin; diltiazem; quinidine; plasma; tissue; concentration; drug interaction; oral administration; repetitive dosing; mice

INTRODUCTION
Diltiazem, which is one of Ca-antagonists,1) has been used extensively in Japan for antianginal and antihypertensive purpose, and is often used in combination with digoxin. Some Ca-antagonists such as verapamil2–4) and nifedipine5,4) have been reported to increase serum digoxin levels as well as quinidine6–7) in man. However, there are few reports about the effects of diltiazem on digoxin serum levels.

In this study, to evaluate the possible interaction between diltiazem and digoxin, mice were chosen for a screening test animal and repeatedly administered digoxin alone or digoxin with diltiazem and the steady-state plasma and tissue digoxin levels were measured. And the interaction between digoxin and diltiazem was elucidated by comparing with that between digoxin and quinidine, which interaction is well known in man6–7) and some animals.8–12)

MATERIALS AND METHODS
Drugs and Dosage Forms — Digoxin (DX) purchased from Sigma Chemical Co. was dissolved in ethanol and diluted with distilled water to make 5 and 10 μg/ml solution (final ethanol concentration: 2.5 and 5 v/v%, respectively). Quinidine sulfate (QD) was of JPX grade (Hoei Yakuko Co., Ltd.) and was dissolved in distilled water to make 2 and 5 mg/ml solution. Diltiazem hydrochloride (DTZ) was obtained in a tablet form (Herbesser®, Tanabe Seiyaku Co., Ltd.). The tablets were ground and suspended in distilled water to contain 1 and 3 mg/ml of DTZ.

Animals and Treatments — Male ddY mice weighing 25–35 g were used. The mice were fed with a commercial diet (Oriental Yeast Co., Ltd.) and allowed access to water ad libitum. Four experiments were carried out depending on the doses of DX and co-administered drugs as shown in Table I. In every experiment, mice were administered DX solution alone, or DX with co-administered drug solution or suspension (total volume 20 ml/kg) in stomach two times daily for 5 d. On day 6 (16 h after the last dose), mice were anesthetized with ether. The
blood was withdrawn from the inferior vena cava, placed in a heparinized tube and centrifuged at 2500 rpm for 15 min to obtain the plasma. After the mice were perfused with saline by the left ventricular injection, the brain, heart, liver, and kidney were removed, weighed and frozen at −20°C until assay.

Serum Protein Binding — Pooled serum was obtained from 10 mice in each group treated the same as Exp. 4. The serum free fraction of DX was determined by an ultrafiltration method using MPS-1 apparatus (Amicon, YMT membrane). Parent serum sample (0.5 ml) was applied to the MPS-1, and the ultrafiltrate was collected by centrifugation at 2000 × g for 10 min. Aliquots from the ultrafiltrate and parent serum sample were diluted with human plasma and DX was assayed. The serum free fraction of DX was calculated after correction for the recovery of DX in the ultrafiltrate in the absence of protein.

Digoxin Assay — The plasma and tissue DX concentrations were measured in duplicate by radioimmunoassay, using a commercial I RIA-kit (Digoxin RIA bead®, Abbott Lab., Ltd.). Tissue DX was extracted by the method of Jogestrand. The recoveries of DX from the brain, heart, liver, and kidney at the concentration of 2 ng/ml were 105.8 ± 7.89, 100.3 ± 8.09, 103.7 ± 8.45, and 119.4 ± 8.99 (mean ± S.D., n = 5) respectively. The assay showed no cross-reaction with DTZ and with QD.

Data Analysis — Student’s t-test was used to evaluate the level of statistical significance. All results were expressed as mean ± S.E.

RESULTS
Plasma Digoxin Concentrations
Fig. 1 shows the plasma digoxin concentrations (PDC) after 5 consecutive days administration of 0.1 mg/kg daily dose of DX (Exp. 1 and 2 in Table I). In Exp. 1, DTZ (20 mg/kg daily) increased PDC from 0.51 ± 0.04 to 0.66 ± 0.09 ng/ml, as QD (40 mg/kg daily) elevated PDC to 1.00 ± 0.13 ng/ml (p < 0.01). Although doses of DTZ and QD were increased to 60 and 100 mg/kg respectively, PDC was not increased substantially (in Exp. 2).

Fig. 2 shows PDC after 0.2 mg/kg of DX administration (Exp. 3 and 4 in Table I). In Exp. 3, DTZ (20 mg/kg) and QD (40 mg/kg) seemed to enhance PDC. In Exp. 4, DTZ (60 mg/kg) and QD (100 mg/kg) elevated PDC significantly from 2.32 ± 0.45 to 4.18 ± 0.62 and 5.44 ± 0.66 ng/ml, respectively.

Serum Protein Binding
In vivo serum protein binding in control, DTZ and QD group was 64.6 ± 3.16, 65.9 ± 1.89 and 67.6 ± 4.13% (mean ± S.D., in 5 repeated measurements). No significant change in the protein binding of DX was observed in the presence of DTZ or QD.

Tissue Digoxin Concentrations
The DX concentrations in brain, heart, liver, and kidney in Exp. 4 are shown in Fig. 3. DTZ as well as QD significantly increased the DX con-

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<th>TABLE I. Daily Dose (mg/kg) of Digoxin (DX), Diltiazem Hydrochloride (DTZ), and Quinidine Sulfate (QD) in Mouse Experiment</th>
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centrations in brain and liver, and tended to rise
the DX concentrations in heart. However, in
kidney, no change was observed with DTZ or
QD.

**Digoxin Tissue/Plasma Concentration Ratios**
The distribution of DX to each of the tissues
in Exp. 4 was indicated as tissue/plasma (T/P)
ratio in Fig. 4. In control group, the T/P ratios

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**FIG. 1.** Effects of DTZ and QD on Plasma Digoxin Concentrations in Mice after Administration of DX (0.1 mg/kg Daily) for 5 d
Values represent mean ± S.E. (n = 8–10). a) Significantly different from control values (p < 0.01).

**FIG. 2.** Effects of DTZ and QD on Plasma Digoxin Concentrations in Mice after Administration of DX (0.2 mg/kg Daily) for 5 d
Values represent mean ± S.E. (n = 8–10).
Significantly different from control values: a) p < 0.05, b) p < 0.01.
FIG. 3. Effects of DTZ (60 mg/kg Daily) and QD (100 mg/kg Daily) on Tissue Digoxin Concentrations in Mice after Administration of DX (0.2 mg/kg Daily) for 5 d
Values represent mean ± S.E. (n = 8–10). Significantly different from control values: a) p < 0.01, b) p < 0.001.

FIG. 4. Effects of DTZ (60 mg/kg Daily) and QD (100 mg/kg Daily) on the Tissue/ Plasma (T/P) Ratios of Digoxin in Mice after Administration of DX (0.2 mg/kg Daily) for 5 d
Values represent mean ± S.E. (n = 8–10). a) Significantly different from control values (p < 0.05).
were greater than 1 in all four tissues, suggesting the tissue binding in all. QD decreased the T/P ratios in brain, heart, and kidney. DTZ also showed the same tendency as QD. In liver, there was a apparent difference between the effects of DTZ and QD on the T/P ratio, that is, DTZ increased the T/P ratio while QD did not.

DISCUSSION

It has been reported that co-administration of QD\(^6\)–\(^7\) or some Ca-antagonists such as verapamil\(^2\)–\(^4\) and nifedipine\(^2\),\(^4\) increased PDC in man. Our present results indicate that the QD also increases PDC in mice as reported in man\(^5\)–\(^7\) and some animals.\(^8\)–\(^12\) The guinea pig\(^8\) and the chicken\(^12\) are proposed as a model animal for DX–QD interaction in humans. The effects of QD on DX concentrations in plasma and some tissues in this mice study were the same as those in the guinea pig\(^8\) and the chicken,\(^12\) therefore mice were thought useful for a screening test animal for DX–DTZ interaction.

DTZ, one of Ca-antagonists, also increased PDC in the same experiments (Fig. 1 and 2). However, the tendency to increase PDC with DTZ seemed to be less than that with QD. Belz et al.\(^4\) reported that PDC was more increased with QD than with verapamil or nifedipine in man.

To elucidate the relationship between the degree of interaction and relative doses of DX and co-administered drugs, four dose-level experiments were performed (Table I). Although DTZ and QD tended to increase PDC in all four experiments, certain dose dependency could not be observed (Fig. 1 and 2).

The most likely mechanisms about DX–QD interactions are (1) displacement of DX from tissue binding sites,\(^5\),\(^8\)–\(^10\),\(^14\),\(^15\) (2) the interference with renal DX excretion,\(^5\),\(^8\),\(^14\),\(^16\) and (3) inhibition of extrarenal elimination\(^5\),\(^8\),\(^15\),\(^17\) by QD.

Effects of DTZ and QD on DX distribution were investigated in several tissues (Fig. 3 and 4); brain as a part of neural toxicity, heart as a target of cardiac effects, and liver and kidney as the major sites of elimination. The DX concentrations in brain, heart, and liver were increased by co-administration of DTZ or QD. Thus, both DX cardiac effects and neural side effects might be enhanced by co-administration of DTZ. Since percent increases of PDC were relatively higher than those of tissue DX, the T/P ratios were decreased in brain, heart, and kidney with DTZ. These decreased T/P ratios indicate the displacement of DX at tissue binding sites by DTZ, because the protein binding of DX in serum showed no significant change in the presence of DTZ. The reason for the increased liver T/P ratio with DTZ was uncertain, but it might be associated with high bile excretion of DTZ in mice.\(^18\)

If the increase in PDC with DTZ were due solely to the lowered tissue DX binding, DX concentrations would be reduced in the tissues. The experimental results, however, showed that the tissue DX concentrations were enhanced, and this DX increase in the tissues is not due to the alteration of the free fraction of DX in serum. Therefore the mechanisms of PDC increase would include not only displacement but also enhanced absorption and/or reduced elimination by DTZ co-administration.

Since marked species variation\(^19\) is known about DX disposition, the present results in mice are not directly applicable to man. The pharmacokinetic studies on interaction between DX and DTZ in human volunteers are in progress.

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