EFFECT OF DIGOXIN ON PLASMA CLEARANCE AND ANTICOAGULANT EFFECT OF WARFARIN IN RATS

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The possible effects of digoxin on the elimination and anticoagulant action of warfarin were examined in rats. The pharmacokinetic parameters of warfarin after a single i.v. (1.2 mg/kg) or repeated oral coadministration with anticoagulant (0.6 mg/kg on day 1, thereafter 0.3 mg/kg) and digoxin (50 µg/kg) were not significantly different as compared with those in the group treated with warfarin alone. However, prothrombin complex activity (PCA) following coadministration with the diuretic was significantly and relatively rapidly recovered as compared with that in the warfarin group. The amounts of warfarin extracted by liver 2 and 6 h after a single i.v. dosing or 3 and 8 h after repeated oral dosing in the coadministered group were significantly decreased as compared with those in the group receiving warfarin alone. The renal function (renal plasma flow rate (RPF) and glomerular filtration rate) in the group coadministered with digoxin was significantly higher than that in the group receiving warfarin alone. On the other hand, the fraction of warfarin bound to BSA or rat plasma and the plasma water were little changed in the presence of digoxin. These results suggest that a pharmacological interaction, the decrease in the anticoagulant action, is induced between warfarin and digoxin coadministered.

Keywords—warfarin; digoxin; elimination; anticoagulant effect; hepatic uptake; renal function; protein binding; drug interaction

INTRODUCTION

Safe therapy with anticoagulant depends to a large degree on the drug region, although many reports on alterations of the activity of anticoagulants such as warfarin by other drugs have appeared in the literature.\(^1\)\(^-\)\(^5\)\)\) Anticoagulants are commonly used in combination with other drugs such as diuretics, cardiac glycosides and tranquilizers in order to improve a variety of symptoms accompanied with thromboembolic disorders. We have previously reported that when a high dose of furosemide is administered concomitantly with warfarin, the diuretic causes the displacement of warfarin from plasma protein binding sites, and consequently changes its anticoagulant effects.\(^6\)\) It is suggested that diuretics may reduce the response to anticoagulants,\(^7\)\(^-\)\(^8\)\) possibly by increasing their excretion.\(^8\)\) On the other hand, it is assumed that cardiotonic glycosides may reduce the response to anticoagulants by relieving edema and congestion.\(^9\)\) However, the detailed reports and the mechanism on the interaction of warfarin and digoxin have not been presented, and the clinical significance of such a reaction is uncertain.

In this study, in attempt to clarify the mechanism of the interaction between warfarin and digoxin, the effects of the glycoside on the pharmacokinetics, anticoagulant action, hepatic uptake of warfarin and renal function were investigated in detail in rats, in addition to estimating the effect of digoxin on the in vitro protein binding of the anticoagulant.

EXPERIMENTAL

Materials — 1) Animals: Male Wistar rats weighing 250–300 g were used throughout the study. The animals had free access to food (MF diet, Oriental Yeast Co., Ltd.) and water before and during the experiment. 2) Drug: Potassium
Warfarin-Digoxin Interaction

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warfarin (warfarin, J.P. grade) and digoxin were obtained from Eisai Co., Ltd. and Aldrich Chemical Co. (Milwaukee, Wisconsin, USA), respectively. Sodium p-aminohippurate injection and sodium thiosulfate injection were purchased from Daiichi Pharm. Co., Ltd. and Banyu Pharm. Co., Ltd., respectively. Coumatetralil, an internal standard for gas liquid chromatography (GLC), was a gift from Nihon Tokushu Noyaku Seizo Co., Ltd. Pentfluorobenzyl bromide, thromboplastin (Bac- to-Thromboplastin) and bovine serum albumin (BSA, fraction V) were purchased from Tokyo Chemical Ind. Co., Ltd., Difco Laboratories (Detroit, Michigan, USA) and Sigma Chemical Co., Ltd. (St. Louis, Missouri, USA), respectively. All other chemicals used were of special grade.

Treatment of Animals — Warfarin and digoxin were administered as a solution in saline, but digoxin was dissolved in a small amount of ethanol and diluted with saline. Intravenous (i.v.) and oral administrations were carried out in volumes of 0.1 ml/100 g and 0.4 ml/100 g respectively to animals. The animals were divided at random into 2 groups, each consisting of 4—7 rats. In a single i.v. administration, animals were treated with either warfarin (1.2 mg/kg) alone or warfarin and digoxin (50 µg/kg). In repeatedoral administration, animals were treated with every other day administration of warfarin from day 1 to day 5 (0.6 mg/kg on day 1, thereafter 0.3 mg/kg) and for 5 days with daily administration of saline (warfarin group) or digoxin, 50 µg/kg (warfarin-digoxin group). After the final treatment, 0.3 ml blood was collected at arbitrary intervals from tail vein in a sodium citrate-treated syringe. The plasma was separated immediately by centrifugation at 1400 × g for 10 min.

Determination of Warfarin Concentration in Plasma — Warfarin concentration in plasma and samples containing the drug were determined by the GLC10 and fluorescence11 methods, respectively, as described in a previous paper.8)

Measurement of Prothrombin Complex Activity — Prothrombin complex activity (PCA) of plasma samples was measured by the one-stage- prothrombin time method of Quick.12)

Determination of Warfarin Concentration in Liver — Animals were fasted for 12 h prior to experiment. Liver homogenate was prepared in 0.25 M sucrose—50 mM phosphate buffer, pH 7.4, 2 and 6 h after a single i.v. dose or 3 and 8 h after the final treatment in repeated oral administration. Warfarin concentration in the homogenate was determined by the GLC method described in a previous report.6)

Protein Determination — Protein concentration was determined by the method described by Lowry et al.13 with bovine serum albumin as a standard. Protein concentration in plasma was determined after centrifugation of blood collected.

Hematocrit Measurement — The hematocrit value was determined by the centrifugation at 12000 rpm for 5 min.

Determination of p-Aminohippuric Acid and Sodium Thiosulfate in Plasma — Animals were treated with a single i.v. or repeated oral administration of warfarin alone or in combination with digoxin. Sodium p-aminohippurate (30 mg/rat) or sodium thiosulfate (95.56 mg/kg), separately, was injected intravenously 2 h after a single i.v. dose or 3 h after the final treatment in repeated oral administration. Both p-aminohippuric acid (PAH) and sodium thiosulfate in plasma were determined according to the method of Brun.14)

In Vitro Binding of Warfarin to Rat Plasma and BSA — Equilibrium dialysis was used to assess protein binding of warfarin to undiluted rat plasma and BSA.15) Drug concentrations were 0.2 mM for warfarin and 0.02-1 µM for digoxin. BSA (0.05 mM) was dissolved in 0.067 M phosphate buffer, pH 7.4. The general approach and technique of this study were the same as described previously.6)

Pharmacokinetic and Statistical Analysis — The area under the plasma drug concentration-time curve (AUC) after administration was determined by the trapezoidal rule up to the last sampling point. The AUC beyond the last observed plasma concentration (Cn) was extrapolated.
according to $C_0/k_{el}$, where $k_{el}$ is the elimination rate constant. The data following a single i.v. dose of warfarin were analyzed by using a one-compartment open model. The pharmacokinetic parameters were calculated by nonlinear least-squares regression analysis, according to the same equations as described previously. Renal plasma flow rate (RPF) and glomerular filtration rate (GFR) were calculated according to the following equations: $RPF = V_{PAH} \cdot \ln 2/t_{1/2}$, $GFR = V_{STS} \cdot \ln 2/t_{1/2}$, where $V_{PAH}$ and $V_{STS}$ are the apparent volume of distribution of PAH and sodium thiosulfate, respectively, and $t_{1/2}$ is the elimination half-life of each reagent. The means of all data are presented with their standard deviation (mean ± S.D.). Student’s $t$-test was utilized to determine a significant difference between the groups, $p < 0.05$ being taken as the minimum level of significance.

RESULTS

**Plasma Concentrations of Warfarin after a Single i.v. Administration of Warfarin or Warfarin and Digoxin**

As shown in Fig. 1, the plasma decay curve of warfarin could be described by a single exponential term following a rapid i.v. injection of the drug (1.2 mg/kg) alone. Thus, it seems that the disposition of warfarin fits to the one-compartment open model. When coadministered with the diuretic, the plasma concentration of warfarin also declined according to a single first-order function. Some pharmacokinetic parameters calculated are summarized in Table I. With digoxin (50 μg/kg), the elimination rate constant of warfarin was slightly increased, but not significant, as compared with that in the group treated with warfarin alone. There was no significant difference in other pharmacokinetic parameters between the warfarin and coadministration groups.

**Plasma Concentrations of Warfarin after Repeated Oral Administration of Warfarin Alone or in Combination with Digoxin**

The plasma concentrations of warfarin after repeated oral treatment with the drug with or without digoxin are shown in Fig. 2. In all groups received warfarin alone and warfarin plus digoxin, the mean maximum plasma concentration of warfarin was attained at 6—9 h after oral administration. No statistically significant difference was observed in all pharmacokinetic parameters between the warfarin and coadministration groups, although there was a tendency to increase the elimination of warfarin in the latter group, as shown in Table II.

**PCA Following Administration of Warfarin Alone or in Combination with Digoxin**

The time course of anticoagulant effect of warfarin following a rapid i.v. injection and repeated oral administration of warfarin alone or in combination with digoxin are shown in Figs. 3A and B, respectively. In a rapid i.v. dose, there was no significant difference in PCA between both groups up to 36 h after administration, while, in the group coadministered with digoxin PCA was markedly recovered beyond 48 h. The PCA at 72 h after administration in the group treated with the combined drugs was 65.5 ± 15.3% of the con-

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**FIG. 1. Semilogarithmic Plots of Plasma Warfarin Concentration after a Single i.v. Administration of Warfarin Alone or in Combination with Digoxin**

Dose: warfarin 1.2 mg/kg, digoxin 50 μg/kg body weight, i.v. ●; warfarin alone, ○; warfarin-digoxin. Each point represents the mean ± S.D. of 4—6 rats. a) $p < 0.01$; compared with warfarin alone.
TABLE I. Pharmacokinetic Parameters of Warfarin after a Rapid i.v. Injection of Warfarin Alone or in Combination with Digoxin

<table>
<thead>
<tr>
<th></th>
<th>$k_d$(h$^{-1}$)</th>
<th>$t_{1/2}$(h)</th>
<th>$V_d$(l/kg)</th>
<th>$Cl$ (ml/kg/h)</th>
<th>$AUC$ (µg·h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin alone</td>
<td>0.0236±0.0047</td>
<td>30.28±6.08</td>
<td>0.158±0.010</td>
<td>3.69±0.52</td>
<td>334.3±43.7</td>
</tr>
<tr>
<td>Warfarin-digoxin</td>
<td>0.0250±0.0053</td>
<td>28.60±5.83</td>
<td>0.167±0.011</td>
<td>4.16±0.83</td>
<td>297.1±58.6</td>
</tr>
</tbody>
</table>

$t_{1/2}$; elimination half-life, $V_d$; apparent volume of distribution, $Cl$; total clearance. Each value represents the mean ± S.D. of 4—6 rats.

trol, whereas PCA in the warfarin group was 15.6±4.7% ($p<0.001$). In the case of repeated oral administration, PCA in the group dosed in combination with digoxin was remarkably recovered as compared with that in the warfarin group at all sampling points.

**Hepatic Uptake of Warfarin**

The amounts of warfarin extracted by liver 2 and 6 h after a single i.v. dose and 3 and 8 h after repeated oral administration of warfarin alone or in combination with digoxin are summarized in Table III. In both single i.v. and repeated oral dose groups coadministered with digoxin, the mounts of warfarin extracted by liver were significantly decreased as compared with those of warfarin group (15−22% decrease). This suggests that the coadministration with digoxin might produce a partial decrease in the inhibition of coagulation factor production by warfarin in liver.

**Effect of Digoxin on Renal Function and Plasma Water**

The plasma decline of PAH and sodium thiosulfate in the group treated with warfarin alone or in combination with digoxin are measured and the former is shown in Fig. 4. In both single i.v. and repeated oral dose groups, the RPF (29.65±1.37 for a single i.v. dose and 26.02±0.54 ml/kg/min for repeated oral dose) after coadministration with digoxin was significantly enhanced as compared with that in the group receiving warfarin alone (23.52±2.38 for a single i.v. dose and 21.35±3.48 ml/kg/min for repeated oral dose, $p<0.05$, respectively), and the GFR was also a tendency to increase in repeated dose group (18.54±2.87 for warfarin alone and 22.18±1.10 ml/kg/min for warfarin-digoxin). In order to elucidate the effect of digoxin on the excretion of plasma water, the protein concentrations in plasma and hematocrit value after drug treatment (a single i.v. dose) were determined. As shown in Table IV, plasma protein concentrations of the rats treated with warfarin alone were not

![Figure 2. Semilogarithmic Plots of Plasma Warfarin Concentration after Repeated Oral Administration of Warfarin Alone or in Combination with Digoxin](image)

The animals were treated for 5 d with daily dosing of digoxin (50 µg/kg) and with every other day dosing of warfarin (on day 1; 0.6 mg/kg, and days 3 and 5; 0.3 mg/kg, respectively). ●; warfarin alone, ○; warfarin-digoxin. Each point represents the mean ± S.D. of 4—5 rats.
TABLE II.  Pharmacokinetic Parameters of Warfarin after Repeated Oral Administration of Warfarin Alone or in Combination with Digoxin

<table>
<thead>
<tr>
<th></th>
<th>$k_{el}(h^{-1})$</th>
<th>$t_{1/2}(h)$</th>
<th>$V_d(l/kg)$</th>
<th>$Cl$ (ml/kg/h)</th>
<th>$AUC$ (µg·h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin alone</td>
<td>0.0199 ± 0.0056</td>
<td>36.85 ± 10.95</td>
<td>0.119 ± 0.003</td>
<td>2.36 ± 0.62</td>
<td>244.8 ± 86.2</td>
</tr>
<tr>
<td>Warfarin-digoxin</td>
<td>0.0217 ± 0.0044</td>
<td>32.75 ± 5.83</td>
<td>0.119 ± 0.011</td>
<td>2.57 ± 0.47</td>
<td>188.0 ± 40.6</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 4–5 rats.

FIG. 3. Effect of Warfarin Administered Either Alone or in Combination with Digoxin on Prothrombin Complex Activity of Rats

Prothrombin complex activity was measured by the method of Quick after administration of the drugs. (A) a rapid i.v. injection, (B) repeated oral administration. ●; warfarin alone, ○; warfarin-digoxin. Each point represents the mean ± S.D. of 4–5 rats. a) $p < 0.001$ and b) $p < 0.01$ respectively compared with warfarin alone.

changed, while in the warfarin-digoxin group the concentrations were trending toward increase only slightly after treatment, but not significant. No significant differences were also found in the hematocrit values between both groups after the same i.v. treatment (44.1–46.1% for warfarin
TABLE III. Hepatic Uptake of Warfarin after a Single i.v. and Repeated Oral Administrations of Warfarin Alone or in Combination with Digoxin

<table>
<thead>
<tr>
<th>Warfarin concentration (ng/g liver weight)</th>
<th>Single i.v.</th>
<th>Repeated p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
<td>6 h</td>
</tr>
<tr>
<td>Warfarin alone</td>
<td>74.54±5.22</td>
<td>75.88±3.02</td>
</tr>
<tr>
<td>Warfarin-digoxin</td>
<td>58.37±4.19</td>
<td>64.53±6.54</td>
</tr>
</tbody>
</table>

Liver sample was collected 2 and 6 h after a single i.v. dose and 3 and 8 h after repeated administration. Each value represents the mean ± S.D. of 4−5 rats. 

a) \( p < 0.003 \) and b) \( p < 0.05 \) respectively compared with warfarin alone.

The present study showed that the coadministration of digoxin in both single i.v. and repeated oral dose groups did not induce significant effect on the disposition of warfarin from plasma, with the exception of a slight decrease in a few pharmacokinetic parameters (Figs. 1 and 2, Tables I and II). However, PCA following coadministration with the diuretic was significantly recovered beyond about 2 d after a single i.v. dosing (Fig. 3A). In repeated oral dose group, the recovery effect by digoxin was much extreme and there was significant difference in PCA between the warfarin and coadministration groups at all sampling points (Fig. 3B). The similar results were also obtained after repeated oral dosing of warfarin and trichloromethiazide (0.5 mg/kg/d, for 5 d) (data not shown). These results suggest that anticoagulant activity of warfarin disappeared relatively rapidly after coadministration with the diuretics or digoxin. From the results, it is also deduced that the information obtained from the plasma decay curves of anticoagulants induce an incorrect interpretation on anticoagulant effect of the drugs when the anticoagulants are dosed in combination with diuretics or digoxin. The mechanism of significant recovery in the PCA after coadministration with digoxin was not explained satisfactorily on the basis of the results obtained, but the recovery was partly ascribed to

diuretics and cardiotonic glycosides. It is suggested that diuretics and cardiotonic glycosides may antagonize the response to anticoagulants. However, the mechanism and clinical significance of the interaction is uncertain. It is therefore worthy to clarify the mechanism of interactions between anticoagulant and digoxin.

DISCUSSION

Since patients receiving anticoagulants frequently have concurrent disease requiring drug treatment, the question of drug interactions with anticoagulants is common and often important. A variety of drugs seems to potentiate or interfere significantly with the activity of anticoagulants,

resulting in disappointing and sometimes hazardous responses. It is suggested that diuretics and cardiotonic glycosides may antagonize the response to anticoagulants. However, the mechanism and clinical significance of the interaction is uncertain. It is therefore worthy to clarify the mechanism of interactions between anticoagulant and digoxin.
TABLE IV.  Plasma Protein Concentrations after a Single i.v. Administration of Warfarin Alone or in Combination with Digoxin

<table>
<thead>
<tr>
<th></th>
<th>Rats body weight (g)</th>
<th>Before treatment</th>
<th>After 2 h</th>
<th>After 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin alone</td>
<td>267 ± 2</td>
<td>63.72 ± 1.69</td>
<td>63.40 ± 1.30</td>
<td>62.35 ± 4.57</td>
</tr>
<tr>
<td>Warfarin-digoxin</td>
<td>267 ± 5</td>
<td>63.16 ± 3.37</td>
<td>64.12 ± 4.56</td>
<td>64.00 ± 2.96</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 4 rats.

FIG. 4.  Semilogarithmic Plots of Plasma Concentration after a Single i.v. Injection of p-Aminohippuric Acid to Drug-Treated Rats

30 mg of p-aminohippuric acid was injected to rats. (A) a single i.v. dose, (B) repeated p.o. dose. ● ; warfarin alone, ○ ; warfarin-digoxin. Each point represents the mean ± S.D. of 4 rats. a) p < 0.05; compared with warfarin alone.

the decreased warfarin concentrations in liver (Table III), possibly due to the enhanced efficiency of the liver to produce prothrombin and other coagulant factors. This may be explained by the potent diuretic action of digoxin, likely by the enhanced excretion of free warfarin. The assumption is supported by a fact that the RPF value was significantly enhanced in the warfarin-digoxin group as compared with that in the warfarin group (Fig. 4), and by the report that cardiotonic glycosides induce an increase in renal blood flow,\(^{17}\) in addition to the fact that the renal
clearance of drugs should be directly proportional to the unbound drug concentration. O'Reilly and Aggeler show that coadministration of chlorothalidone results in a significant reduction in hypoprothrombinemic effect without significant differences in plasma levels of warfarin in man.\(^1\)\(^{18}\) The authors also propose that a decrease in plasma water attributable to the diuretic action may induce concentration of clotting factors.\(^1\)\(^{18}\) However, it would be not reasonable to assume that the concentration of coagulation factors is produced in liver, since the decrease in plasma water was hardly observed after coadministration of warfarin and digoxin (Table IV). Although we designed the determination of free warfarin in urine of both groups, it was not able to estimate the concentrations by the present methods as described in Methods because of many hindrances in urine.

It is of very interest that the significant difference was not observed in the plasma concentrations and pharmacokinetic parameters of warfarin between the warfarin and coadministration groups, in spite of the dramatic difference in PCA, in particular, PCA in repeated oral dose groups. Apparently no significant effect of digoxin on the elimination of warfarin from blood (Figs. 1 and 2) may be partly explained by that since warfarin is a highly protein binding drug (98.5–99.8%),\(^{19}\)\(^{20}\) the increased excretion of the free fraction of warfarin in plasma by digoxin would not apparently contribute to the total plasma levels. We could not demonstrate the hypothesis, as the free warfarin concentration in each sample was not determined because of a little volume (0.3 ml) of blood collected. This assumption, however, is supported by the results that the plasma concentrations of warfarin after coadministration were only slightly, but not significantly, lowered as compared with those after the drug alone in both single and repeated dose groups (Figs. 1 and 2).

The protein binding of warfarin to rat plasma and BSA was not changed in the presence of digoxin. The result implies that the increased PCA after coadministration with digoxin was not due to the displacement of warfarin from its binding sites, in contrast to ethacrynic acid.\(^{21}\)

In conclusion, the combination of warfarin and digoxin did not affect the pharmacokinetic parameters of warfarin, however, the combination markedly decreased the anticoagulant effect of warfarin. The combination with digoxin may enhance the efficiency of the liver to produce prothrombin and other coagulation factors, probably due to a decrease in the hepatic extraction of warfarin. The concentration of clotting factors in liver due to a decrease in plasma water was little involved in the increased efficiency. Since the anticoagulant effect of warfarin in vivo is markedly affected when digoxin is coadministration with the anticoagulant, it is necessary to take much care in the treatment with both warfarin and digoxin.

REFERENCES


