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Effect of Itraconazole on the Pharmacokinetics of Digoxin in Guinea Pigs

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ABSTRACT: The effect of itraconazole (ITZ) on the pharmacokinetics of digoxin (DGX) was investigated in guinea pigs. The plasma concentrations of DGX in guinea pigs following treatment with ITZ (20 mg/kg intraperitoneally (ip)) after intravenous (iv) administration of DGX (0.125 mg/kg) were significantly higher than in the controls. The percentage cumulative excretion (6.9%) of DGX in bile of the ITZ-treated group up to 6 h after administration of DGX was significantly reduced ($p < 0.05$) compared with 10.5% in the control. The percentage cumulative excretion (3.1%) of DGX in urine of the ITZ-treated group up to 6 h after administration was also one third that in the controls. The total, biliary, renal and metabolic clearances for DGX in the ITZ-treated group were significantly reduced, while the V_{dss} was unaffected. The plasma unbound fraction of DGX (ranged 47–58%) in the ITZ-treated group was generally similar to that in the controls (50–57%). The blood-to-plasma concentration ratio of DGX (range 1.28–1.42) in the absence of ITZ did not change in the presence of ITZ. Based on these results, the pharmacokinetic interaction between DGX and ITZ may be due not only to a reduction in the renal clearance but also to a reduction in the metabolic clearance of DGX by ITZ. Copyright © 1999 John Wiley & Sons, Ltd.

Key words: digoxin; itraconazole; pharmacokinetic interaction; renal clearance; metabolic clearance; guinea pigs

Introduction

Itraconazole (ITZ) is a triazole antifungal drug with activity against a variety of pathogens including candida, aspergilus and other species. This drug is a potential inhibitor of the cytochrome P-450 isozyme, CYP 3A4, and can seriously interact with some substrates of CYP 3A4, such as cyclosporin, terfenadine and triazolam [1–3]. In addition, important interactions between ITZ and digoxin (DGX) leading to elevation of serum DGX concentrations in association with signs and symptoms of DGX toxicity, such as lethargy, persistent nausea and blurred vision, have been the subject of several clinical reports [4–8]. However, the mechanism of the interaction leading to toxic DGX levels is not clear.

In this paper, the authors have tried to reproduce the pharmacokinetic interaction between ITZ and DGX in guinea pigs, in order to elucidate the mechanism of this interaction.

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Materials and Methods

Chemicals

DGX was obtained from commercial sources as a solution containing 0.25 mg/mL of DGX for injection (Digosin®, Dai Nippon Pharmaceutical Co., Osaka, Japan). ITZ was kindly supplied by Janssen-Kyowa Co. (Tokyo, Japan). Sodium pentobarbital was obtained from Dai Nippon Pharmaceutical Co. (Nembutal®) and polyethylene glycol 200 (PEG) was from Wako Pure Industries Ltd. (Tokyo).

Animals

Male Hartley strain guinea pig (Japan Laboratory Animals Co., Tokyo) weighing 250–350 g were used. Animals were housed in a well-ventilated cage at 20–22°C for at least 1 week before experiments. Food (a solid feed MF, Orient Yeast Co., Tokyo) and water were given *ad libitum*.

Preparation of Solution or Suspension for Administration

A digoxin injection containing DGX (0.25 mg/mL) solution was diluted two times with physiological

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saline and used for administration. Eighty-five percent PEG aqueous solution containing ITZ (10 mg/mL) was prepared by the method of Mikami *et al*. [9]. After ITZ was dissolved in 12 M aqueous HCl solution, the PEG solution was added, and then the pH of the mixture was adjusted to 4–6.5 with 6 M NaOH aqueous solution

Drug Administration and Sample Collection

Polyethylene canulas were inserted into the carotid artery and vein, common bile duct and gall bladder under sodium pentobarbital (30 mg/kg intraperitoneally (ip)) anesthesia. After surgery, the animals were left to return to consciousness. A dose (20 mg/kg) of ITZ was then given ip (ITZ-treated group). At 1 h, a dose (0.125 mg/kg) of DGX was administered intravenously (iv) through the venous cannula. Blood samples (0.15 mL) for DGX assay were collected through the arterial cannula in heparinized tubes at 1, 5, 10, 15, 30 min, and 1, 2, 3, 4 and 6 h after administration. Bile was collected 0–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–5 and 5–6 h, and urine was collected 0–2, 2–4 and 4–6 h after administration of DGX. Plasma (50 μL) was separated from blood by centrifugation at 10000 rpm for 2 min, and assayed as described below. Bile and urine samples were stored at −20°C until analysis. As controls, animals were pretreated ip with 85% PEG solution (dosing volume 2 mL/kg) and, 1 h later, the same dose of DGX was administered.

To determine the plasma concentration of ITZ, another experiment was performed, because the large plasma volume (1 mL) required for assay. The same dose of DGX as above was administered, and at 1 h after ip administration, a dose (20 mg/kg) of ITZ was administered ip to two animals. Blood samples (2 mL) were collected just before administration of DGX, and 2 and 6 h after administration of DGX. Plasma (1 mL) was separated from blood as described above and stored at −20°C until analysis.

Measurement of Plasma–Protein Binding

At 1, 5, 15, 30, 60 or 180 min after iv administration of DGX to both groups of animals, blood (2.5 mL) was collected through the venous cannula. Individual animals contributed two blood samples. Plasma (1.2 mL) was separated from blood by centrifugation. After 0.2 mL plasma was taken for assay of the total DGX concentration, the remaining portion (1 mL) was placed in a disposable ultrafiltration device (MP-3 centrifree; Amicon Corporation, Denvers, MA) and then centrifuged at 3000 rpm for 5–7 min at room temperature, and 0.1 mL of the filtrate (unbound concentration) was assayed.

Measurement of Blood-to-Plasma Concentration Ratio

After blood was collected from three guinea pigs and pooled, 1.15 mM phosphate buffer (pH 7.4) (volume 50 μL) containing DGX was added to 1 mL blood to give a concentration of 5–140 ng/mL. The sample was incubated at 37°C for 20 min. One portion (50 μL) was used for the determination of the drug concentration in blood, and the other was centrifuged to give plasma. Plasma and blood samples were assayed as described below.

Drug Assay

Plasma, blood and the ultrafiltrated solution were diluted to various degrees with blank plasma, and concentrations of DGX were determined by a fluorescence polarization immunoassay (TDx, Abott). Urine and bile were diluted ten times with blank plasma, and assayed by the TDx method. There was no cross-reactivity found in the determination of DGX concentrations involving ITZ. The detection limit for DGX was 0.2 ng/mL. Whenever the concentration of DGX in plasma was determined, control plasma of known concentration (0.75, 1.5, 3.5 ng/mL) was assayed to check the reproducibility of the assay. Plasma concentrations of ITZ were determined by the high performance liquid chromatographic method of Woestenborghs *et al*. [10]. The assay of the hydroxy metabolite of ITZ in plasma was carried out by Mitsubishi Chemical BTL Co. (Tokyo), since the authors could not obtain this authentic compound.

Pharmacokinetic Analysis

The plasma concentration profiles of DGX after iv administration were fitted to Equation (1) using a nonlinear least-squares program MULTI [11]:

$$C_t = P \cdot \exp(-\pi \cdot t) + A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t) \quad (1)$$

where C_t is the plasma concentration of drug at time t; P, A and B are ordinate intercepts, π, α and β are the corresponding first-order disposition rate constants. The elimination half-life ($t_{1/2}$) in the β-phase was calculated from $0.693/\beta$. The volume of distribution at steady-state (V_{dss}) was calculated by a conventional equation [12] using three exponentials. The area under the plasma drug concentration–time curves (AUC) after administration up to the last sampling point was calculated by the trapezoidal rule, and the AUC beyond the last observed plasma concentration (C_{ob}) was extrapolated using C_{ob}/β. The total body clearance (Cl_{tot}) was calculated from dose/AUC. The biliary clearance (Cl_{Bi}) of DGX was obtained by dividing $AUC_{0-360\ min}$ by the cumulative biliary excretion up to 360 min after dosing, and the renal clear-

ance (Cl_R) by dividing AUC_{0-360} min by the cumulative urinary excretion. The metabolic clearance (Cl_M) was calculated from Equation (2).

$$Cl_M = Cl_{tot} - Cl_{Bi} - Cl_R \quad (2)$$

Statistical Analysis

Statistical analysis was performed by paired analysis of variance with $p = 0.05$ as the minimum level of significance.

Results

Effects of ITZ on Biliary and Urinary Excretion of DGX

The time courses of the plasma concentration of DGX in the control and the ITZ-treated groups are shown in Figure 1. The plasma concentration of DGX at 15 and 30 min and 6 h in the ITZ-treated group after iv administration of DGX was significantly higher than that in the control. The plasma concentration of ITZ gradually increased over 6 h after dosing (Figure 1). Although the hydroxy metabolite of ITZ was detectable, its concentration was low (less than 100 ng/mL) at any sampling time (data not shown). The biliary excretion rate and percentage cumulative biliary excretion of DGX in both groups after iv administration of DGX are shown in Figure 2.

The biliary excretion rate of DGX in the ITZ-treated group after administration was significantly lower than that in the control. The percentage cumulative excretion of DGX in the ITZ-treated group until 6 h after administration of DGX was 6.9%, and the value was significantly smaller compared with 10.5% in the control. As shown in Figure 3, the urinary excretion rate of DGX after iv administration of DGX was also significantly reduced by co-administration of ITZ. The percentage cumulative urinary excretion (3.1%) of DGX in the ITZ-treated group until 6 h after administration was one third that in the control group. Table 1 summarizes the pharmacokinetic parameters of DGX in both groups of guinea pigs. Although the V_{dss} was not affected by co-administration with ITZ, the values of Cl_{tot}, Cl_{Bi}, Cl_R and Cl_M were significantly reduced.

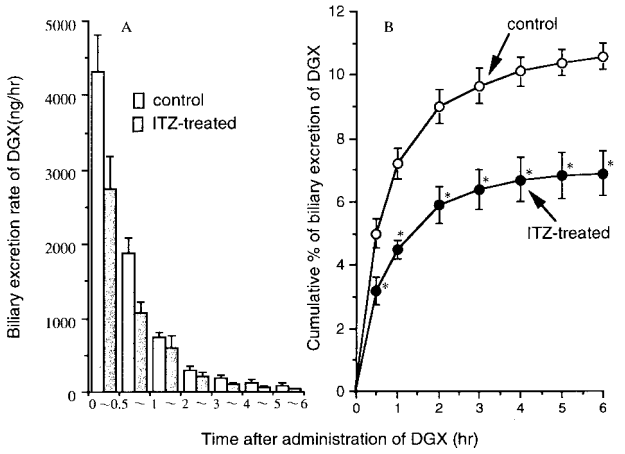

Figure 2. Biliary excretion rate (panel A) and percentage cumulative of biliary excretion of DGX (panel B) in control and ITZ-treated groups after iv administration of DGX (0.125 mg/kg) in guinea pigs ($n = 4$ in each group). The results are means ± S.D. * $p < 0.05$

Effects of ITZ on Plasma–Protein Binding and Blood-to-Plasma Concentration Ratio of DGX

The total plasma concentration of DGX in both groups was within the range 4.1–104.2 ng/mL, while the unbound fraction of DGX in the control was relatively constant (range 50–57%). The un-

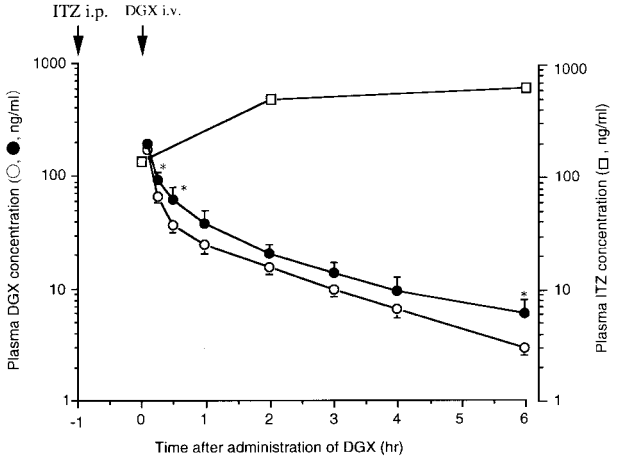

Figure 1. Time courses of plasma concentrations of DGX in control (open symbols) and ITZ-treated (closed symbols) groups after intravenous administration of DGX (0.125 mg/kg) in guinea pigs ($n = 4$ in each group). The results are means ± S.D. * $p <$ 0.05. ITZ (square symbols) was given ip (20 mg/kg dose, $n = 4$). The results are mean values

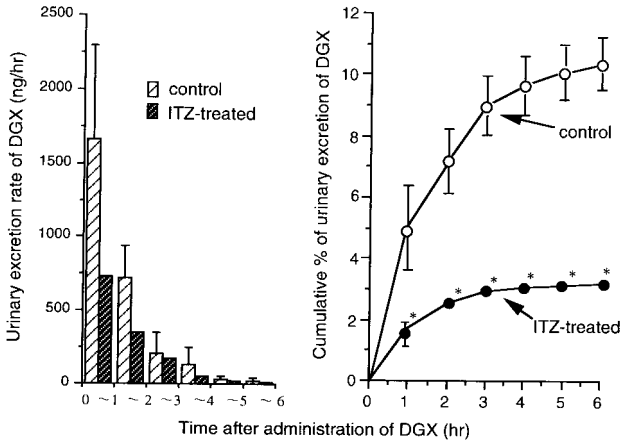

Figure 3. Urinary excretion rate (panel A) and percentage cumulative of urinary excretion of DGX (panel B) in control and ITZ-treated groups after iv administration of DGX (0.125 mg/kg) in guinea pigs ($n = 4$ in each group). The results are means ± S.D. * $p < 0.05$

Table 1. Pharmacokinetic parameters of digoxin (DGX) after iv administration of DGX in control and itraconazol (ITZ)-treated guinea pigs[a]

Parameter	Control	ITZ-treated
P (ng/mL)	272.4 ± 24.4	304.7 ± 53.1
π (min^{-1})	0.158 ± 0.020	0.234 ± 0.078
A (ng/mL)	19.3 ± 4.0	81.8 ± 28.0*
α (min^{-1})	0.0354 ± 0.0247	0.0246 ± 0.0036
B (ng/mL)	31.8 ± 7.8	25.6 ± 0.3
β (min^{-1})	0.0065 ± 0.0006	0.0041 ± 0.0006**
$t_{1/2\beta}$ (min)[b]	107.1 ± 8.7	170.6 ± 22.7**
V_{dss} (L/kg)	1.88 ± 0.21	1.77 ± 0.26
Cl_{tot} (mL/min/kg)	17.41 ± 1.78	11.75 ± 2.72*
Cl_R (mL/min/kg)	1.22 ± 0.29	0.43 ± 0.09**
Cl_{Bi} (mL/min/kg)	1.97 ± 0.21	0.94 ± 0.29**
Cl_M (mL/min/kg)	15.86 ± 1.31	10.38 ± 2.34*

[a] Digoxin was administered iv at dose of 0.125 mg/kg 1 h after ip administration of 85% PEG solution (control) or itraconazol 20 mg/kg (ITZ-treated) to guinea pigs ($n = 4$ in each group). The results are means $\pm$ S.D.
[b] $t_{1/2\beta}$, eliminatin half-life.
* $p < 0.05$, ** $p < 0.01$, indicates results are significantly different from the control.

bound fraction in the control was almost the same as that in the ITZ-treated group (47–58%).

The blood-to-plasma concentration ratio in the presence of ITZ was within the range 1.28–1.38, and this was not significantly different from that (1.28–1.42) in the absence of ITZ.

Discussion

Clinically, there have been reports [4–8] that taking DGX have developed elevated serum concentration of DGX in association with DGX toxicity after the addition of ITZ to their treatment. In the present study, the authors were able to reproduce this elevation in the plasma concentration of DGX in guinea pigs. Marzo and Ghirardi reported [13] that DGX was mainly eliminated via the liver in guinea pigs, and the unchanged DGX in both bile and urine was 5–8%, the more polar metabolites were also in the bile (73%) and urine (31%) as were the less polar metabolites (bile 11%, and urine 56%). The findings on the excretion of unchanged DGX after administration alone are essentially in agreement with the results of Marzo and Ghirardi [13]. The findings on the excretion of DGX suggest that ITZ reduced both the biliary and urinary excretion of DGX (Figures 2 and 3). On the other hand, DGX was reported in man to be excreted largely in urine as the unchanged form after iv administration of DGX, and its urinary recovery was 60–80% [14,15]. Sheiner *et al*. [16] reported that the total body, renal and metabolic clearances of DGX were 2.90 ± 1.0, 1.64 ± 0.50 and 0.790 ± 0.460 mL/min/kg (mean $\pm$ S.D.), respectively. Judging from the human data, the elevation of the plasma concentrations of DGX in man after the addition of ITZ may be explained partly by reduced renal clearance rather than metabolic clearance.

Recently, Tanigawara *et al*. [17] showed that in transport studies using human *p*-glycoprotein expressed in a porcine kidney epithelial cell line, LLC-PK1, that DGX is a substrate of *p*-glycoprotein, and the mechanism of clinically important drug interactions, such as digoxin–quinidine, was elucidated. Also, it has been reported [18,19] that ITZ could be a substrate of *p*-glycoprotein. Based on these studies on *p*-glycoprotein, the authors strongly suggest that the renal excretion of DGX may be inhibited by ITZ. Although these results showed that the biliary excretion of DGX may be inhibited by ITZ, it is unclear whether this inhibition was due to interaction with *p*-glycoprotein present in the common bile duct or not.

Both the plasma–protein binding and permeability into the blood cell of DGX were unchanged following co-administration of ITZ. This may be partly reflected in the unchanged V_{dss} (Table 1). On the other hand, the serum concentration of DGX has been reported to be increased, when quinidine was given to patients [20] or guinea pigs [21] receiving DGX. The reduction in Cl_{tot} and the volume of distribution of DGX accounts for this elevation. Sato *et al*. [22] suggested that the reduced tissue-to-plasma concentration ratio of DGX in tissues such as heart, liver, muscle and brain, is due mainly to the inhibition of the tissue distribution of DGX by quinidine. Evidence has also been produced [23] showing that quinidine was capable of reducing the affinity for DGX of cardiac glycoside receptors on purified Na^+, K^+-ATPase in guinea pigs. From these studies, it is unlikely that ITZ may inhibit the tissue distribution of DGX, since the V_{dss} of DGX was unchanged by ITZ.

Following the present results, further studies of the mechanism of the reduced biliary, urinary and metabolic clearance of DGX following co-administration of ITZ are in progress in guinea pigs.

In conclusion, it was shown that the pharmacokinetic interaction between DGX and ITZ may be due not only to a reduction in renal and urinary clearances but also in the metabolic clearance of DGX by ITZ.

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