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Comparative Study of Increased Plasma Quinidine Concentration in Rats with Glycerol- and Cisplatin-induced Acute Renal Failure

Yuki IZUWA, Jun-ichi KUSABA, Mizuki HORIZUCHI, Tetsuya AIBA*, Hiromu KAWASAKI and Yuji KUROSAKI

Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

Summary: A comparative study of altered plasma concentration of quinidine in rats with glycerol- and cisplatin-induced acute renal failure (ARF) was conducted with quinidine used as a positively charged and liver-metabolized therapeutic compound. Although apparent total body clearance of quinidine decreased to 68 and 48% of the normal value in glycerol- and cisplatin-induced ARF rats, respectively, its distribution decreased only in glycerol-induced ARF rats. The plasma unbound fraction of quinidine decreased in glycerol-induced ARF rats, which was not observed in cisplatin-induced ARF rats. The plasma level of α1-acid glycoprotein (AGP) increased in glycerol-induced ARF, but not in cisplatin-induced ARF rats. It is therefore conceivable that the plasma concentration of positively charged and liver-metabolized compounds generally increases due to hepatic elimination suppressed as renal function decreases, but the pharmacokinetic impact of suppressed hepatic elimination is occasionally difficult to observe in some ARF model rats since it may be blurred by the influence of increased plasma AGP level.

Keywords: α1-acid glycoprotein; acute renal failure; cisplatin; glycerol; quinidine

Introduction

Decreased renal function is well recognized as a factor altering pharmacokinetics and drug dispositions, and it frequently leads to increase in the plasma concentration of therapeutic compounds undergoing renal excretion. Displacement of protein-bound compounds from their binding sites on the serum protein often occurs accompanying decrease in renal function, due to accumulated uremic toxins, such as indoxyl sulfates and furan dicarboxylic acids. These toxins largely affect interactions between drugs and serum albumin, and, therefore, the displacement and resultant increase in distribution are mainly observed for acidic or negatively charged compounds, including meloxicam, phenytoin and tolbutamide.

Altered drug dispositions with decreased renal function have been conclusively investigated in the case of acidic compounds. In contrast, for basic or positively charged compounds, reported findings on altered pharmacokinetics with decreased renal function are relatively inconclusive. That is, the plasma concentrations of some compounds such as disopyramide, ropivacaine, and quinine, have been shown to increase in patients with renal failure, while, for others, such as metoclopramide and mefloquine, plasma concentrations seem to remain unaltered in such patients. Various animal experiments have been conducted to find how decreased renal function affects the plasma concentration and disposition of these compounds, but ambiguity still remains. Some experiments involving lidocaine and quinidine demonstrated that plasma concentrations increased as renal function decreased, while others observed no detectable alterations with propranolol. The precise reason for this ambiguity is unknown, but seems related to differences in clinical conditions of the patients and those in experimental methods utilized to induce renal failure in laboratory animals.

Various compounds commonly used for circulatory and neuropsychiatric diseases are generally categorized as positively charged and hepatically eliminated compounds and the clinical necessity of these compounds in-
creases with advancing age. It is therefore meaningful to characterize their altered pharmacokinetics and disposition under disease conditions. In this study, with quinidine, whose renal elimination is less than 3% of the dosed amount and which was selected as a representative compound, we performed a comparative study on its altered pharmacokinetics in rats with glycerol- and cisplatin-induced acute renal failure to clarify why pharmacokinetic alterations accompanying decreased renal function have been diversely observed.

**Materials and Methods**

**Materials:** Quinidine sulfate, tolvatamide and cis-diamineplatinum (II) dichloride (CDDP, cisplatin) were purchased from Sigma (St. Louis, MO, U.S.A.). Purified rabbit anti-rat α1-acid glycoprotein (AGP) antibodies were purchased from Life Diagnostics Inc. (West Chester, PA, U.S.A.). Glycerol and other chemicals used were of the finest grade available.

**Animals:** Male Wistar rats (210–330 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were housed at 20–25°C and 40–50% humidity with a 12-h light/dark cycle and allowed free access to a standard laboratory diet and water prior to the experiments. All animal experiments were performed in accordance with the guidelines for animal experimentation of Okayama University.

**Induction of acute renal failure in rats:** Acute renal failure (ARF) was experimentally induced by two methods. One was with an intramuscular injection of glycerol and the other was an intraperitoneal injection of cisplatin. To induce ARF with glycerol, rats were injected with 50% glycerol saline solution (10 ml/kg) in the left and right leg muscles after 24-h water deprivation. The rats were mainly used in experiments 24 h after the glycerol injection. Some were used 12 or 48 h after injection, if necessary. To induce ARF with cisplatin, rats were injected with 0.1% cisplatin saline solution (5 ml/kg) in the abdominal cavity. The rats were mainly used in experiments 72 h after the cisplatin injection, and some were used 36 or 96 h after injection.

Some ARF as well as normal rats were used to collect plasma specimens. After being anesthetized with sodium pentobarbital (50 mg/kg i.p.), a rat was fixed on its back. A midline incision was carefully made and blood was drawn from the aorta. The blood was centrifuged to collect plasma. The specimens were pooled and stored at −40°C until used to measure creatinine concentration by the Jaffe reaction to confirm ARF development. The pooled specimens were also used for the protein binding assay and Western immunoblot analysis as described later.

**Pharmacokinetic study on ARF rats:** After being anesthetized, a rat was fixed on its back and its femoral artery and jugular vein were cannulated with polyethylenetubing (SP-31, Natsume Seikakusho, Tokyo, Japan) for drug administration and blood collection, respectively. Quinidine was dissolved in 0.9% sodium chloride at a concentration of 6.25 mg/ml and administered at a dose of 12.5 mg/kg. As a reference, the effects of decreased renal function on the pharmacokinetics of a negatively charged and heparically eliminated therapeutic compound were examined in this study using tolvatamide as a representative compound, if necessary. It was dissolved in 0.9% sodium chloride at a 10 mg/ml and administered at a dose of 10 mg/kg. After the administration, blood samples were collected at scheduled times, and plasma specimens were obtained by 10-min centrifugation (12,000 × g, 4°C). A 100-μl of plasma specimen was obtained from a 250-μl of the blood sample collected. The plasma specimens were subjected to HPLC determination, as described later.

Plasma drug concentrations were characterized in a model-dependent manner, using the following equation:

\[ Cp(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \]  \hspace{1cm} (1)

where \( Cp(t) \) is plasma drug concentration at time \( t \). Parameters in the equation, \( A, \alpha, B, \) and \( \beta \), were estimated with a nonlinear, least-squares method. Apparent total plasma drug clearance (\( CL \)) was calculated by dividing the administration dose (\( D \)) by the area under the concentration-time curve (\( AUC \)) obtained from equation below. The apparent volume of distribution (\( V' \)) was obtained by dividing \( D \) by plasma drug concentration at time zero. The volume of distribution at a steady state (\( V_{dss} \)) was obtained as follows:

\[ AUC = \int_0^{\infty} Cp(t)dt \]  \hspace{1cm} (2)

\[ MRT = \int_0^{\infty} t \cdot Cp(t)dt/AUC \]  \hspace{1cm} (3)

\[ V_{dss} = CLt \cdot MRT \]  \hspace{1cm} (4)

**In vitro protein binding study:** Quinidine was dissolved in a pooled plasma specimen at a concentration of 0.3–6 μg/ml. A part of this on was used to confirm the total drug concentration with HPLC. A total of 400 μl of the solution was applied to the Ultrafree®-MC micropartition ultrafiltration device (Millipore, Billerica, MA, U.S.A.), and then centrifuged (1,500 × g, 10 min) at room temperature to obtain the filtrate. The filtrate was weighed and then applied onto HPLC to determine the unbound drug concentration. The unbound drug fraction was calculated from unbound and total drug concentrations. As a reference, the protein-unbound fraction of tolvatamide was evaluated in the same manner as above. Tolvatamide was used at a concentration of 20–120 μg/ml.

The protein binding properties of quinidine and tolvatamide were characterized assuming that they bind to
the plasma protein at a single binding site.\(^7\) The relationship between protein-bound drug concentration (\(Cb\)) and unbound drug concentration (\(Cf\)) is given as follows:

\[
\frac{1}{Cb} = \frac{Kd}{Bm} \left( \frac{1}{Bm} \right) + \frac{1}{Cf}
\]

(5)

where \(Bm\) and \(Kd\) are the concentration of the total drug binding site and dissociation constant, respectively. In this study, these parameters were calculated based on the slope and intercept of the regression line in double reciprocal plots, taking error into consideration.

**Evaluation of plasma AGP:** Plasma AGP level was evaluated by Western immunoblot analysis.\(^{22,23}\) Briefly, the plasma specimen was mixed with Tris solution containing sodium lauryl sulfate and glycerol and applied to SDS-polyacrylamide (15%) gel at 5.0 \(\mu\)g protein per lane. The gel was subjected to 150-min electrophoresis followed by transfer to a nitrocellulose membrane. After the membrane was subjected to overnight blocking with 4% skim milk containing 0.4% Tween 20, it was incubated for 1 h with anti-rat AGP antibody diluted at 1:5,000. The migration pattern was visualized with a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, U.S.A.). The visualized band signals were analyzed based on densitometric reading. The band signal of the plasma specimen from ARF rats was normalized using the band signal of a specimen from normal rats.

**Analytical methods:** Quinidine and tolbutamide were determined by HPLC with an octadodecyl silica column (4 \(\mu\)m, 4.6×150 mm, Synergi Fusion-RP, Phenomenex, Torrance, CA, U.S.A.). The plasma specimen was mixed with methanol followed by vigorous agitation and centrifugation (12,000 \(\times\) g, 10 min). The obtained supernatant was applied onto HPLC. For quinidine determination, the mobile phase was prepared from methanol and 0.05% phosphoric acid (22:78), and used at a flow rate of 1.0 ml/min. Quinidine was spectrophotometrically determined at excitation and emission wavelengths of 350 and 450 nm, respectively. The detection limit of quinidine was 2 ng/ml. Tolbutamide determination differed for glycerol- and cisplatin-induced ARF rats. That is, quinidine concentration in glycerol-induced ARF rats was generally higher than in normal rats, while alteration in cisplatin-induced ARF rats was not noticeable until 90 min after administration (Fig. 1B). Differences in concentrations were reflected by pharmacokinetic parameters. The distribution of quinidine significantly decreased in glycerol-induced ARF rats, but remained unchanged in cisplatin-induced ARF rats (Table 1).

**Protein binding properties of quinidine in glycerol- and cisplatin-induced ARF rats:** Change in distribution was thought related to alteration in quinidine plasma unbound fraction and thus its protein binding was examined in vitro using plasma specimens obtained from glycerol- and cisplatin-induced ARF rats. In contrast with negatively charged tolbutamide, the protein binding affinity of quinidine increased in glycerol-induced ARF rats (Fig. 2), causing the slope in double reciprocal plots in glycerol-induced ARF rats to be significantly less than in normal rats (0.045 ± 0.002 vs. 0.108 ± 0.006, respectively; \(p < 0.05\) (Fig. 2B). In spite of significant differences in slope values, binding could not be properly derived in this analysis due to large deviations; the calculated values in glycerol-induced ARF rats were 0.13 ± 0.03 \(\mu\)g/ml for \(Kd\) and 2.9 ± 0.8 \(\mu\)g/ml for \(Bm\), and those in normal rats were 1.6 ± 2.4 \(\mu\)g/ml and 14.8 ± 22.6 \(\mu\)g/ml, respectively. Binding affinity in cisplatin-induced ARF rats seemed to decrease from normal values being opposite to those for glycerol-induced ARF rats (Fig. 2B).

**Plasma AGP levels in glycerol- and cisplatin-induced ARF rats:** As shown in Figure 3, plasma AGP levels were examined in glycerol- and cisplatin-induced ARF rats. In glycerol-induced ARF rats, plasma AGP significantly increased from the normal level when measured at 12, 24, and 48 h after glycerol treatment (Figs. 3A and 3B). AGP started to decrease at 24 h after treatment, while serum creatinine concentration, a marker of impaired kidney function, was maximal at this point (Fig. 3B). In cisplatin-induced ARF rats, no increase in plasma AGP was observed despite increase in serum creatinine (Figs. 3B and 3D).

**Discussion**

The total plasma concentration of quinidine increased in experimentally induced ARF rats, but altered plasma concentration profile varied depending on the ARF induction method. As shown in Figure 1B, plasma concentrations of quinidine in glycerol-induced ARF rats

Quinidine Pharmacokinetics in ARF Rats

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Fig. 1. Plasma concentration-time profiles of tolbutamide (panel A) and quinidine (panel B) observed after intravenous administration in normal and ARF rats.

In panel A, the tolbutamide profiles in normal and glycerol-induced ARF rats are indicated with ‘○’ and ‘●’, respectively. In panel B, the quinidine profiles in normal, glycerol-, and cisplatin-induced ARF rats are shown with ‘○’, ‘●’, and ‘▲’, respectively. Data are presented as the mean ± S.E. of 3–5 experiments. *p < 0.05: significantly different from the corresponding value in normal rats.

Table 1. Pharmacokinetic parameters of tolbutamide and quinidine in normal and ARF rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal rats</th>
<th>Glycerol-induced ARF rats</th>
<th>Cisplatin-induced ARF rats</th>
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<tbody>
<tr>
<td>Tolbutamide</td>
<td></td>
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<tr>
<td>AUC (µg·hr/ml)</td>
<td>713 ± 79</td>
<td>507 ± 59</td>
<td>—</td>
</tr>
<tr>
<td>CLt (l/hr)</td>
<td>0.015 ± 0.002</td>
<td>0.021 ± 0.002</td>
<td>—</td>
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<tr>
<td>Vd (l/kg)</td>
<td>0.079 ± 0.003</td>
<td>0.114 ± 0.006</td>
<td>—</td>
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<tr>
<td>Vdss (l/kg)</td>
<td>0.150 ± 0.003</td>
<td>0.177 ± 0.007</td>
<td>—</td>
</tr>
<tr>
<td>Quinidine</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AUC (µg·hr/ml)</td>
<td>3.99 ± 0.10</td>
<td>6.24 ± 0.77</td>
<td>8.80 ± 1.25</td>
</tr>
<tr>
<td>CLt (l/hr)</td>
<td>3.14 ± 0.08</td>
<td>2.11 ± 0.29</td>
<td>1.49 ± 0.24</td>
</tr>
<tr>
<td>Vd (l/kg)</td>
<td>2.91 ± 0.28</td>
<td>1.76 ± 0.23</td>
<td>2.49 ± 0.22</td>
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<tr>
<td>Vdss (l/kg)</td>
<td>6.58 ± 0.38</td>
<td>4.93 ± 0.47</td>
<td>7.04 ± 0.40</td>
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* Data are shown as the mean ± S.E. of 3–5 experiments.
† Experiments were not performed.
‡ *p < 0.05: significantly different from the corresponding value in normal rats.

were generally higher than in normal rats, reflecting a decrease in distribution (Table 1). In cisplatin-induced ARF rats, the alteration in quinidine was noticeable only in its terminal phase and distribution remained unchanged (Table 1). This suggests that hepatic elimination of quinidine is suppressed in cisplatin-induced ARF rats. Hepatic elimination in glycerol-induced ARF rats, was thought altered in the same manner as observed in cisplatin-induced ARF rats. However, the suppressed quinidine elimination in glycerol-induced ARF rats could not be detected in this study probably since the effect of the suppressed hepatic elimination on the plasma quinidine profile was blurred by the influence of altered plasma AGP level in glycerol-induced ARF rats. Since positively charged compounds like quinidine bind to plasma AGP with a high affinity (Figs. 2 and 3), increased plasma AGP level in glycerol-induced ARF rats is likely to affect quinidine pharmacokinetics sufficiently to interfere with the detection. Depending on whether in the plasma AGP level changes, the effects of acute renal failure on the plasma concentrations of positively charged compounds was seen to vary.

In this study, increased plasma AGP was observed only in glycerol-induced ARF rats (Fig. 3). Since AGP is an acute phase protein and its plasma level increases in response to inflammation, observed differences in AGP increase is probably explained by the presence or absence of inflammation. In the case of glycerol-induced ARF rats, 50% glycerol is intramuscularly injected to osmotically and/or physically collapse muscle tissues, causing myoglobin to be released from the injection site. The released myoglobin accumulates in the kidney via the blood stream and impairs kidney function. Cisplatin-induced ARF rats are prepared by intraperitoneal injection of a small volume of the nephrotoxic compound cisplatin which accumulates in the kidney and impairs its function, and renal failure occurs. It is therefore likely that intense inflammation occurs with muscular tissue collapse in glycerol-induced ARF rats, while not or less intensively in cisplatin-induced ARF rats. As a result, inflammation-related factors, such as interleukin-6 and tumor necrosis factor-α, are abundantly produced and
Fig. 2. Protein binding of tolbutamide (panel A) and quinidine (panel B) in normal and ARF rats
In panel A, the unbound fractions of tolbutamide examined with plasma specimens from normal and glycerol-induced ARF rats are indicated with ‘○’ and ‘●’, respectively. The regression lines for normal and glycerol-induced ARF rats are shown as solid and dotted lines, respectively. In panel B, the unbound fractions of quinidine for normal, glycerol-, and cisplatin-induced ARF rats are indicated with ‘○’, ‘●’, and ‘◆’, respectively, and their regression lines are shown as solid, dotted and dashed lines, respectively.

Fig. 3. Plasma AGP levels in glycerol- and cisplatin-induced ARF rats
In panels A and C, the representative results from Western immunoblot analysis are shown for glycerol- and cisplatin-induced ARF rats, respectively. In panels B and D, the plasma AGP levels in glycerol- and cisplatin-induced ARF rats are indicated with gray columns. AGP levels in glycerol-induced ARF rats were measured at 12, 24, and 48 hr after glycerol treatment (panel B), and those in cisplatin-induced ARF rats, at 36, 72, and 96 hr after cisplatin treatment (panel D). In both panels, the normal levels for normal rats are shown as open columns. Serum creatinine concentrations measured with pooled specimens from each group are also shown as a line graph. Data are indicated as means ± S.E. of 4–10 experiments, if applicable. *p<0.05: significantly different from the normal value. A significance test was not performed for creatinine concentration.
released from damaged tissues in glycerol-induced ARF rats to stimulate hepatic AGP synthesis.\textsuperscript{27,34} The inflammation decreases the hepatic expression of drug metabolizing enzymes.\textsuperscript{39} Therefore, it is quite likely that intrinsic hepatic quinidine clearance in glycerol-induced ARF rats is reduced from the normal value, though the relationship between inflammation and hepatic drug clearance remains to be clarified.

The decreased quinidine clearance in cisplatin-induced ARF rats is consistent with several reports that the pharmacokinetics of hepatically eliminating compounds change when renal function decreases.\textsuperscript{36,37} Although the pharmacokinetics of hepatically eliminating compounds in cisplatin-induced ARF rats is reduced from the normal value, though the intrinsic hepatic quinidine clearance in glycerol-induced ARF rats is consistent with several reports that the clearance remains to be clarified.

The decreased quinidine clearance in cisplatin-induced ARF rats is consistent with several reports that the pharmacokinetics of hepatically eliminating compounds change when renal function decreases.\textsuperscript{36,37} Although the precise mechanism for decreased clearance is unknown, it seems to involve decreased activity and/or expression of the drug metabolizing enzyme cytochrome P450 3A in the liver of ARF rats.\textsuperscript{25,37,38} It is also probable that uremic toxins, such as indoxyl sulfates and furan dicarboxylic acids, inhibit hepatic quinidine metabolism. Cisplatin is reported to slightly accumulate in the liver and this may affect hepatic function\textsuperscript{39,40} causing change in quinidine pharmacokinetics. Further investigation is necessary to elucidate the effects of ARF on hepatic drug metabolism in cisplatin-induced ARF rats.

This study demonstrates that plasma concentrations of positively charged and hepatically eliminating compounds change in experimentally induced ARF rats, due to hepatic drug elimination suppression; these concentrations vary depending on the presence or absence of increased plasma AGP level. It is necessary to take change in plasma AGP levels into consideration when the effects of ARF on the pharmacokinetics and drug disposition of positively charged and hepatically eliminating compounds are examined.

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