PHARMACOLOGY

The effect of chronic cadmium exposure on the pharmacokinetics of theophylline and ciprofloxacin in rats

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Abstract

Cadmium has been associated with a number of adverse health effects but the impact of those effects on the pharmacokinetics of different drugs has not been investigated. Therefore, the pharmacokinetics of theophylline and ciprofloxacin were studied in cadmium-exposed and control rats (72 rats) following i.p. (6.5 mg/kg) and p.o. (10 mg/kg) administration, respectively. The third-generation offsprings of rats exposed to 100 mg/mL of cadmium chloride in drinking water were used in this study. Following 8 weeks of exposure, animals received the drugs as a single dose. Blood samples were withdrawn at different time-points and the plasma concentrations of both drugs were analyzed by HPLC. The pharmacokinetic parameters of theophylline and ciprofloxacin were altered significantly in the cadmium-exposed animals. For theophylline, a statistically significant increase ($p<0.0001$) in $C_{\text{max}}$ (69\%) and $AUC_{0-\infty}$ (68\%) of theophylline in the cadmium-exposed rats as compared to the control were observed. A corresponding significant ($p<0.0001$) reduction of 41\% in clearance ($CL/F$) of theophylline was detected in the exposed group. Neither the half-life nor the mean residence time (MRT) showed any significant change due to the exposure to cadmium. For ciprofloxacin, no significant difference was seen in the $C_{\text{max}}$ of the exposed group as compared to the control animals. However, a delay in $T_{\text{max}}$ was observed in the exposed group (from 0.16 (± 0.003) to 0.37 (± 0.14) h). A small, but significant increase in $t_{1/2}$ ($p<0.05$) was detected (1.74 (± 0.25) vs. 1.45 (± 0.12) h). A significant reduction ($p<0.05$) of $CL/F$ from 30.54 (± 1.9) to 24.01 (± 3.81) mL/min/kg was seen in the treated group. The current investigation showed that chronic exposure to cadmium could have a very significant impact on altering the pharmacokinetic parameters of various drugs. Therefore, in cadmium-polluted areas, dose adjustments and drug monitoring, especially for drugs with a narrow therapeutic window, should be carried out.

Keywords: Cadmium; Theophylline; Ciprofloxacin; Pharmacokinetics; Renal failure

Introduction

Cadmium (Cd) is a non-essential trace element that is used in various chemical forms in metallurgical and other industrial processes. Oral exposure to Cd is the major route in the non-industrially exposed individual, where food consumption constitutes the main environmental source of Cd for the non-smoking general population [1].

After the Gulf War, clam samples collected near Kuwait contained higher levels of several metals (Cd,
Cu, Ni, Pb, and V) in 1991 compared to the levels in 1985 [2]. Recently, de Mora et al. [3] have demonstrated that very high concentrations of Cd (up to 195 μg/g) in the liver of some fish from southern Oman may result from food-chain bioaccumulation of elevated Cd levels brought into the productive surface waters by upwelling in the Gulf region.

Cigarette smoking is another source of Cd intake for humans, since tobacco plants (Nicotiana spp.) readily absorb Cd from polluted soils. Cd intake by inhalation in the general population averages 0.02 μg/d, but can be as high as 2 μg/d in highly polluted areas. Therefore, human exposure to Cd and Cd compounds may occur in both occupational and environmental settings [1,4], which is a major concern. This metal was designated recently as a human carcinogen and has been linked to respiratory tumors in occupationally exposed populations [5].

Cadmium is transported in blood and distributed widely in the body but accumulates mainly in the liver and kidneys [6]. The Cd burden in the kidneys tends to increase in a linear fashion with age up to 50 or 60 years of age [7]. Cd has a large volume of distribution indicative of the rapid tissue concentration [8]. It is thought that the half-life of Cd is very long, and that accumulation of Cd occurs in the liver and kidneys. The chronic renal effects of Cd are characterized by proximal tubular necrosis and dysfunction, and can occur following chronic oral or inhalation exposure [7]. Signs of liver damage, hypoalbuminemia and metabolic acidosis can accompany Cd intoxication after oral exposure [9].

A recent study by Baker et al. [10] suggested that cadmium may be an inducer of renal and hepatic isozymes (CYP4F2 and CYP2E1 and CYP4F2), and that increased renal CYP4F2 expression may result in cadmium-linked renal tubular dysfunction and high blood pressure. Thus, the question arises of whether human exposure to Cd, with potential hepatotoxicity and nephrotoxicity, would significantly affect the pharmacokinetics of drugs eliminated by these organs, and to what extent? The biological risk from environmental and occupational exposure to pollutants have been well established in different reviews [4,11–13]. However, the effect of environmental and industrial pollution on the pharmacokinetics of different drugs has not been investigated. Nation et al. [11] published a brief communication on the effect of exposure to cadmium in the drinking water on the ethanol pharmacokinetics in rats. Although this communication demonstrated the effect of 60 d of exposure to Cd on ethanol concentration–time profiles, no pharmacokinetic parameter was presented. Thus, we attempted to investigate the impact of physiological changes due to cadmium exposure on the pharmacokinetics of two model drugs; namely, theophylline and ciprofloxacin. These two drugs have different pathways of elimination. Theophylline has a narrow therapeutic window, and it is metabolized extensively by the liver. Hepatic metabolism is the main pathway of elimination, which undergoes parallel first-order and Michaelis–Menten reactions [12,13]. The metabolism may become saturated (non-linear) within the therapeutic range [14,15]. As a result, small increases of dose may result in disproportionately large increases in serum concentration [16]. Ciprofloxacin, one of the antimicrobial quinolones, acts by inhibition of the essential bacterial enzyme DNA gyrase. Quinolones have a high level of activity against a broad spectrum of Gram-negative and -positive bacteria [17]. Ciprofloxacin is an example of a drug that is eliminated by both renal excretion and hepatic metabolism; a mean of 50% of an intravenous dose was found unchanged in urine in 24 h [18]. In the current study, the effect of chronic exposure to cadmium on the pharmacokinetic parameters of two model drugs with different elimination pathways was investigated.

**Materials and methods**

**Materials**

Theophylline and cadmium chloride were purchased from the Sigma Chemical Company (St. Louis, MO, USA). Ciprofloxacin powder was obtained from Miles Pharmaceuticals (West Haven, CT, USA). All other reagents and chemicals were of analytical grade and were used as received.

**Animals**

A total of 72 male Sprague–Dawley rats weighing 200–250 g were used in this study. Half of the rats were the third-generation offspring of mothers exposed to cadmium from the first day of birth and the pups along with the mothers were exposed to cadmium chloride (100 μg/mL) continually in their drinking water for 8 weeks until the day of the study (exposed group). These exposed animals were divided into a theophylline group and a ciprofloxacin group, and each group was divided randomly into three subgroups in three cages (six rats per cage) for different sampling time-points. The other 36 animals were pair-watered with water containing no added cadmium (control group). The control rats were divided into two groups; one for theophylline and the other for ciprofloxacin. Each group was divided randomly into three subgroups (six rats per cage). Animals were fasted for at least 12 h before the experiment but water was available ad libitum throughout the experiment.
On the day of experiment; 18 rats of the cadmium-exposed group and 18 rats of the control group received i.p injection of 6.5 mg/kg of theophylline solution, while the other half received an oral dose (via a gastric tube) of 10 mg/kg of ciprofloxacin suspension (5 mg/mL)

**Blood sampling**

Blood samples (0.5 mL) were collected at time 0, 10, 20, 30, 45 min, 1, 2, 3, 4, 6, 20, and 24 h after dosing from the orbital venous plexus under light halothane anesthesia. Only three or four blood samples were taken from each rat per day to avoid any damage to the eye and to keep the blood volume constant. Each datum point represents the mean of six rats. Plasma samples were separated by centrifugation at 4000 rpm for 15 min and samples were kept at −4 °C pending HPLC analysis.

**Drug analysis**

Concentrations of theophylline were measured using a sensitive HPLC assay [19]. Briefly, 200 μL of rat plasma samples were spiked with 20 ng of the internal standard (500 ng of zidovudine) in 10-mL centrifuge tubes. Then, 50 μL of isopropyl alcohol was added and the tube contents were vortex mixed for 30 s. The drug was extracted with 2 mL of chloroform and vortex mixed at high speed for 1 min. After centrifugation at 1000 rpm for 5 min, the organic layer was evaporated and the residue was reconstituted with 100 μL of the mobile phase (7.5% acetonitrile in 0.2% acetic acid). A 50-μL sample of the solution was injected into the HPLC system for analysis. The column was a Novapak C18 column (3.9 × 150 mm) packed with 5-m spherical particles. The sample run time was 8 min.

For ciprofloxacin, plasma samples (200 μL) were assayed in plasma by HPLC as described [20]. Assays were performed after sample dilution with 0.1 N phosphoric acid. The internal standard was the isopropyl analog of ciprofloxacin. The mobile phase for this system consisted of acetonitrile, methanol and trichloroacetic acid with the pH adjusted to 3 with 1 N sodium hydroxide.

**Data analysis**

All results were expressed as mean ± SE. All pharmacokinetic parameters were calculated by non-compartmental methods [21] using in-house developed BASIC program and RSTRIP, version 5.0, (Micromath Scientific Software, Salt Lake City, UT). The terminal elimination rate constant (λ) was estimated by linear regression analysis of the terminal portion of the log-linear plasma concentration–time profile of a drug. The area under each drug concentration time curve (AUC, μg mL⁻¹ h) to the last datum point were calculated by the linear trapezoidal rule and extrapolated to time infinity by the addition of C_last/λ, where C_last is the concentration of the last plasma sample measured. The area under the first moment (AUMC) was determined by RSTRIP using the rules followed for the AUC calculation. The mean residence time (MRT) was estimated from MRT = AUMC/AUC and elimination half-life (t1/2) was calculated from the terminal elimination rate constant using the formula t1/2 = 0.693/λ. The apparent total clearance (Cl/F) and apparent volume of distribution at steady state (Vss/F) were calculated using non-compartmental equations where, Cl/F = (Dose/AUC) and Vss/F = (Cl×MRT). All data were compared using ANOVA, Student’s t-test, and Tukey’s tests. Differences were assumed to be statistically significant when p<0.05.

**Results**

Since the effect of chronic exposure to Cd on the pharmacokinetics of drugs is not documented in the literature, the current study was designed to address this lack of information. The pharmacokinetic parameters mean ± SE were calculated for both theophylline and ciprofloxacin in control and Cd-exposed (Cd-Ex) animals, and are presented in Tables 1 and 2, respectively. The plasma concentration–time profiles of theophylline are depicted in Fig. 1. The concentrations of theophylline in Cd-Ex rats increased significantly, reaching the maximum concentrations (C_max) at 0.33 h as compared to 0.6 h in control rats (p<0.001).

Increases of about 69% and 68% in C_max and the area under the curve (AUC), respectively, were observed in the Cd-Ex animals as compared to control rats. As a result, a significant difference was detected in the apparent clearance (Cl/F), as Cl/F was decreased from 1.8(±0.18) to 1.1(±0.1) mL/min/kg. Therefore, theophylline Cl/F is reduced by 41% due to exposure to Cd, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control rats</th>
<th>Cd-exposed rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_max (μg/mL)</td>
<td>10.2 ± 1.1</td>
<td>17.3 ± 2.2a</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>0.6 ± 0.3</td>
<td>0.33 ± 0.1</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>3.2 ± 0.4</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>AUC (μg h/mL)</td>
<td>60.7 ± 6.1</td>
<td>102.2 ± 7.2b</td>
</tr>
<tr>
<td>AUMC (μg h²/mL)</td>
<td>284 ± 36.4</td>
<td>471.7 ± 30.3b</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>4.6 ± 0.2</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>CL/F (mL/min/kg)</td>
<td>1.8 ± 0.2</td>
<td>1.1 ± 0.1b</td>
</tr>
<tr>
<td>Vss/F (L/kg)</td>
<td>0.5 ± 0.1</td>
<td>0.29 ± 0.1b</td>
</tr>
</tbody>
</table>

*aStatistically significant with p<0.05.  
bStatistically significant with p<0.001.
which could result in significant adverse effects in humans, since theophylline is a drug with a narrow therapeutic window. The apparent volume of distribution was reduced significantly after the exposure to Cd (0.294 ± 0.1 vs. 0.5 ± 0.1 L/kg). The simultaneous effect of cadmium on both Cl and V of theophylline resulted in no significant change in the terminal half-life ($t_{1/2}$).

On the other hand, the pharmacokinetics of ciprofloxacin was not altered significantly on exposure to cadmium, but there some changes. Fig. 2 represents the mean plasma concentrations of ciprofloxacin in control and Cd-Ex rats following p.o. administration of ciprofloxacin at 10 mg/kg ($N = 6$).

![Fig. 1. The mean (± SE) plasma concentration–time profiles of theophylline in control and Cd-Ex rats following i.p. administration of theophylline at 6.5 mg/kg ($N = 6$).](image)

![Fig. 2. The mean (± SE) plasma concentration–time profiles of ciprofloxacin in control and Cd-Ex rats following p.o. administration of ciprofloxacin at 10 mg/kg ($N = 6$).](image)

Table 2. Pharmacokinetic parameters (mean ± SE) of ciprofloxacin in control and Cd-treated rats following oral administration of 10 mg/kg of ciprofloxacin suspension ($N = 6$)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control rats</th>
<th>Cd-exposed rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>3.8 ± 0.4</td>
<td>4.5 ± 0.9</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.16 ± 0.01</td>
<td>0.4 ± 0.1$^a$</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>1.45 ± 0.1</td>
<td>1.7 ± 0.3$^a$</td>
</tr>
<tr>
<td>AUC (µg h/mL)</td>
<td>5.5 ± 0.4</td>
<td>7.1 ± 1.3$^a$</td>
</tr>
<tr>
<td>AUMC (µg h$^2$/mL)</td>
<td>11.6 ± 0.7</td>
<td>16.3 ± 2.2$^a$</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>2.03 ± 0.1</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>CL/F (mL/min/kg)</td>
<td>30.5 ± 1.9</td>
<td>24 ± 3.8$^a$</td>
</tr>
<tr>
<td>Vss/F (L/kg)</td>
<td>3.9 ± 0.3</td>
<td>3.1 ± 0.4</td>
</tr>
</tbody>
</table>

$^a$Statistically significant with $p<0.05$.

The Cl/F was decreased significantly from 30.5(± 1.9) to 24(± 3.8) mL/min/kg when animals were exposed to Cd.

**Discussion**

Cd consumption increased significantly during the early 1900s and has continued unchanged. There are major industries and occupations that are associated with potential exposure to cadmium [22]. Despite the fact that occupational exposure was the most prominent form, significant exposure has occurred and is still occurring through the environment.

Most salts of Cd are absorbed poorly from the gastrointestinal tract, only about 5% of the Cd that is ingested is absorbed [23]. However, Cd has a relatively long transit time in the gastrointestinal tract [23]. Therefore, in the present study, there was Cd in the drinking water at all times for the three generations to ensure chronic exposure. Animals used in the current study were part of another study investigating the effect of cadmium on some physiological parameters.

In a study conducted by Hietanen [24], cadmium was administered to rats in water at a concentration equivalent to 250 µg/mL of cadmium for 2 and 8 weeks. The author concluded that cadmium affected the drug biotransformation rates differently in various tissues; mainly liver, kidney and intestine. Baker et al. [10] has implicated cadmium for the expression of nine cytochrome P450 forms in human liver and kidney cortex samples. Multiple linear regressions showed concentrations of cadmium of 0.38 µg/L in the blood to be associated significantly with effects on renal tubules [25]. Also, experimentally induced subchronic and acute cadmium nephrotoxicity was characterized by significant damage to cortical proximal tubules, resulting in reabsorptive and secretory defects [26]. Theophylline is metabolized extensively by cytochrome P4501A2.
(CYP1A2) as the major isozyme in adults [13]; therefore, it seems that exposure to Cd could result in a significant effect on liver isozymes.

The elimination of ciprofloxacin is dependent mainly on the efficiency of the kidney and secondly on the metabolizing enzyme P450. The renal clearance of ciprofloxacin far exceeds the glomerular filtration rate, indicating substantial active tubular secretion. It was shown that administration of probenecid reduced the renal excretion of ciprofloxacin by 50% [27]. The change in ciprofloxacin Cl/F when animals were exposed to Cd could indicate that the effect on ciprofloxacin elimination was not as severe as that observed for theophylline. This may be due to the involvement of different isoenzymes, or the effect of Cd on the kidney was not as intense as that on the liver. It is not clear whether the absorption rate, excretion rate, and critical concentration of Cd in the renal cortex that produces renal dysfunction in the population exposed to high levels of Cd is the same as those in the population exposed to very low levels of Cd. The accumulation of Cd in various organs following acute or chronic administration varied according to the experimental setting [28,29]. With chronic, low-level exposure of humans to Cd, it has been shown that Cd has an extremely long biological half-life (approx. 30 years in the human body) [30]. The metal is distributed mainly to the liver and kidney in humans and other animals [31,32]. Acute exposure to Cd produces liver injury, because of the hepatic accumulation of the metal [33]. On the other hand, chronic administration of Cd commonly results in renal damage [22,34].

Conclusion

A significant effect of chronic exposure to cadmium on the pharmacokinetics of the two selected drugs was demonstrated in the current study. Theophylline Cl/F is reduced by 41% due to the effect of Cd exposure on the liver, which could be hazardous to humans, since theophylline has a narrow therapeutic window. On the other hand, the pharmacokinetics of ciprofloxacin on exposure to cadmium was not altered as drastically as that of theophylline, yet the changes were statistically significant. The wide environmental distribution of Cd might prevent patients from utilizing their therapy by adding more burdens to their eliminating organs and consequently increase the toxicity of the drugs.

References


