Cephaloridine *in Vitro* Toxicity and Accumulation in Renal Slices from Normoglycemic and Diabetic Rats

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Cephaloridine, a cephalosporin antibiotic, is associated with nephrotoxicity in humans (Kabins and Cohen, 1966; Foord, 1975) as well as experimental animals (Atkinson et al., 1974). As a result of several reports of renal damage in patients treated with high doses of cephaloridine. Although cephaloridine is no longer approved for clinical application, cephaloridine continues to be an important investigative tool since the primary target of the diffusion of the antibiotic is the proximal tubule (Silverblatt et al., 1970; Cojocel et al., 1983). Cellular changes associated with cephaloridine include rapid loss of the brush border, increased vacuole formation, and rounding of mitochondria.

The selectivity of cephaloridine for the proximal tubule may be due to the chemical properties of the antibiotic. Cephaloridine attains very high intracellular levels within the proximal tubule because it is actively transported into the cell along the basolateral membrane by the anion transporter and efflux out of the cell along the brush border is very slow since cephaloridine exists as a zwitterion (Welles et al., 1966; Tune, 1972; Tune et al., 1974). As a result of this phenomenon, cephaloridine selectively accumulates in the cortex to concentrations that are much higher than plasma levels (Tune and Fravert, 1980). Consequently, intracellular organelles within the proximal tubule are exposed to higher levels of cephaloridine than other tissues.

Although the exact mechanism of cephaloridine toxicity has not been identified, mitochondrial damage and/or acylation of mitochondrial proteins have been proposed as possible cellular mechanisms of toxicity. Renal cortical mitochondrial function is rapidly diminished by cephaloridine (Tune et al., 1979). The diminished mitochondrial respiration induced by cephaloridine has been suggested to be mediated by acylation of substrate transporters and subsequent reduction of substrate supply to the mitochondria (Tune et al., 1988). Toxicity may also be due to oxidative stress through generation of peroxidative species (Kuo et al., 1983; Cojocel et al., 1985). Kuo and associates (1983) reported a rapid depletion of glutathione and increased lipid peroxidation in Fischer 344 rats following cephaloridine administration. Cephaloridine was also capable of inducing lipid peroxidation directly in renal microsomes (Cojocel et al., 1985) and in renal slices (Goldstein et al., 1986).

Toxicity is apparent following acute administration *in vivo*

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as well as in vitro following cephaloridine exposure of renal slices or tubules. Renal cortical slices exhibit a concentration- and time-dependent decrease in gluconeogenesis and diminished accumulation of organic ions (Goldstein et al., 1986). Diminished sodium-potassium ATPase activity and mitochondrial function are also evident in primary cultures of rat renal cortical epithelial cells exposed to cephaloridine (Smith and Acosta, 1987). Cephaloridine toxicity is apparent in species other than rats. For example, isolated rabbit proximal tubules (Rush and Ponsler, 1991) exposed to cephaloridine experience a rapid decline in mitochondrial function and depletion of ATP. Cephaloridine can also mediate a rapid depletion of glutathione and induction of lipid peroxidation (Rush and Ponsler, 1990) prior to induction of cell leakage of lactate dehydrogenase (LDH).

Earlier studies (Valentovic et al., 1989) observed that cephaloridine in vivo nephrotoxicity was diminished in streptozotocin (STZ) diabetic Fischer 344 rats. Acute administration of cephaloridine in normoglycemic rats was associated with increased kidney weight, elevated blood urea nitrogen concentration, and diminished renal cortical slice accumulation of organic ions. Diabetic animals, however, exhibited comparable values for blood urea nitrogen concentration and renal cortical slice accumulation of organic anions between pair-fed controls and cephaloridine-treated animals. Therefore, preliminary studies established that the streptozotocin diabetic rat is a model that is less susceptible to cephaloridine toxicity. Later studies have determined that regulation of the diabetic state by insulin pretreatment reversed the protection associated with the diabetic state (Valentovic and Ball, 1995). These results support the hypothesis that resistance was conferred by the diabetic state and not streptozotocin. The potential significance of this observation is that understanding the mechanism for reduced toxicity may provide new avenues to avert the renal damage of other compounds that induce oxidative stress or alter mitochondrial function.

The following were the aims of this paper: (a) compare in vitro cephaloridine toxicity between diabetic and normoglycemic animals; (b) determine the contribution of renal accumulation to reduced toxicity in diabetic tissue; and (c) examine whether diabetic tissue is resistant to toxicity due to a higher gluthathione status. In vitro exposure obviated differences in toxicity due to physiological changes in glomerular filtration rate or renal blood flow that are known to occur in diabetes. Renal cortical slices were selected as the in vitro model for all studies. Although the mechanism for reduced toxicity has not been entirely established, the mechanism may be a combination of alterations in renal accumulation and/or cellular changes that protect the diabetic tissue from toxicity. Understanding the mechanism for reduced cephaloridine toxicity in diabetes may provide new information that could have more general applicability in developing new interventions of other nephrotoxic agents.
because of decreased cephaloridine accumulation. These studies were conducted using renal cortical slices in order to remove the confounding factors of physiological changes in renal blood flow and glomerular filtration rate that occur in diabetes. Renal cortical slices from normoglycemic and diabetic animals were incubated for 30 min at 37°C under an oxygen atmosphere and constant shaking (100 cycles/min) with 0–5 mM cephaloridine in a total volume of 3 ml Krebs buffer. The renal cortical slices were removed from the medium weighed and homogenized in water (5 vol/ weight) using a Tekmar homogenizer (Cincinnati, OH). All tissues were homogenized on ice. Cephaloridine was measured using an HPLC method (Pasino et al., 1985). A 300-μl aliquot of renal homogenate was added to 700 μl acetonitrile in order to precipitate the proteins. A Beckman (Columbia, MD) HPLC system with an ISCO variable wavelength detector was used for all analyses. Cephaloridine was quantitated at a wavelength of 254 nm using a Waters C18 μBondapak column. The mobile phase was 20% acetonitrile prepared in 1% acetic acid at a flow rate of 1.5 ml/min. The extraction efficiency was greater than 96%.

**Determination of glutathione.** Total and oxidized glutathione were measured in renal cortical slices incubated with 0 or 5 mM cephaloridine for 15, 30, 60, or 120 min. Renal cortical slices were blotted, weighed, and homogenized in 5% sulfosalicylic acid using a Tekmar homogenizer. The homogenizer probe was rinsed with 500 μl sulfosalicylic acid and a final volume of 1 ml. The homogenate was centrifuged at 10,000g for 10 min at 4°C. Total glutathione was measured according to the procedures described by Griffith (1980). Glutathione levels were expressed as nmoles per gram of tissue.

**Statistical analysis.** Values were reported as means ± SE with all groups containing a minimum of five different animals. Differences within groups were quantitated using an analysis of variance (ANOVA) followed by a Dunnett's at a 95% confidence interval. Differences between groups were determined using a one way analysis of variance followed by a Newman Keuls if multiple groups were present.

**RESULTS**

**Diabetic Status of the Animals**

Diabetes was confirmed in the diabetic animals by glucosuria in excess of 200 mg/dl. Plasma glucose values were 145 ± 10 mg/dl in the normoglycemic group compared to 486 ± 32 mg/dl in the diabetic group.

**Concentration and Time-Dependent Toxicity**

Cephaloridine inhibited pyruvate directed gluconeogenesis in normoglycemic and diabetic tissue. Incubation of renal slices with 2–5 mM cephaloridine produced a concentration-dependent inhibition of gluconeogenesis (Fig. 1), when compared to control, in normoglycemic and diabetic tissue. Cephaloridine induced a rise in LDH leakage only in the normoglycemic group (Fig. 2). A cephaloridine concentration of 4–5 mM was needed to induce LDH leakage relative to vehicle treated tissue. LDH leakage was not elevated in diabetic tissue exposed to 2–5 mM cephaloridine for 120 min. The results indicate cephaloridine toxicity was greater in the normoglycemic than the diabetic group. These results are also in agreement with previous in vivo findings of decreased susceptibility in diabetic groups toward cephaloridine toxicity.

Studies were next conducted to investigate whether the diabetic state delayed the appearance of cephaloridine cyto-

![FIG. 1. Concentration-dependent effect of cephaloridine on gluconeogenesis. Renal cortical slices were incubated for 90 min with 0–5 mM cephaloridine. Glucose formation was stimulated by the addition of 10 mM pyruvate and further incubation for 30 min. Values expressed as milligrams of glucose/gram of tissue. Values represent means ± SE with four to six animals/group. *Different (p < 0.05) from respective 0 mM group.](image)
mulation were not apparent between normoglycemic and diabetic groups when the concentration of cephaloridine was increased to 4 and 5 mM. These results suggest that accumulation was not the primary mechanism for attenuation of toxicity since accumulation differences between normoglycemic and diabetics were not evident at 4–5 mM cephaloridine, a concentration that induced LDH leakage in renal slices from normoglycemic animals.

**DISCUSSION**

Cephaloridine in vitro toxicity was diminished in diabetic relative to normoglycemic tissue. The diabetic group exhibited decreased renal toxicity when comparisons were made as a function of concentration or time of exposure to cephaloridine. These findings were in agreement with in vivo studies (Valentovic et al., 1989) that had characterized a resistance in diabetic animals injected with a toxic dose of cephaloridine. Additional studies later established that the resistance toward cephaloridine in vivo toxicity was revers-

FIG. 2. Concentration-dependent effect of cephaloridine on LDH leakage. Renal cortical slices were incubated with 0–5 mM cephaloridine for 120 min. LDH leakage was expressed as a percentage of total. Values represent means ± SE with four to six animals/group. *Different (p < 0.05) from respective control (0 mM).

diabetic tissue (Table 2). These findings would suggest that the reduced susceptibility in the diabetic model was not due to a higher baseline level of glutathione protecting the diabetic tissue from oxidative stress. Cephaloridine addition to renal slices from normoglycemic animals decreased (p < 0.05) total glutathione levels within 60 min when compared to control values. Cephaloridine addition to the diabetic tissue required an exposure time of 120 min to decrease total glutathione levels relative to control values. The data obtained on glutathione levels indicated that the diabetic group was less susceptible to depletion of glutathione by cephaloridine. Oxidized glutathione levels were not reported as the levels approached the sensitivity of the assay.

**Cephaloridine Accumulation**

Diminished cephaloridine accumulation would be a plausible explanation for the disparity in toxicity between normoglycemic and diabetic tissue. Renal cortical slice accumulation of cephaloridine was increased as a function of concentration in normoglycemic and diabetic tissues (Fig. 4). Cephaloridine accumulation was lower in the diabetic compared to the normoglycemic tissue when renal slices were incubated with ≤2 mM cephaloridine. Differences in accumu-

FIG. 3. Effect of cephaloridine on gluconeogenesis as a function of time. Renal cortical slices were incubated with 4 mM cephaloridine for 15–120 min in the presence of 10 mM pyruvate. Values expressed as milligrams of glucose/gram of tissue. Values represent means ± SE with 4–6 animals/group. *Different (p < 0.05) from respective control (0 mM).
TABLE 1
Effect of Cephaloridine on LDH Release

<table>
<thead>
<tr>
<th>Group</th>
<th>Cephaloridine (mM)</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycemic</td>
<td>0</td>
<td>6.2 ± 0.4*</td>
<td>5.5 ± 0.2*</td>
<td>6.0 ± 0.3*</td>
<td>7.7 ± 0.5*</td>
<td>8.1 ± 0.6*</td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>5</td>
<td>6.8 ± 0.6*</td>
<td>5.8 ± 0.3*</td>
<td>6.0 ± 0.2*</td>
<td>7.5 ± 0.3*</td>
<td>12.8 ± 0.8*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0</td>
<td>4.8 ± 0.2*</td>
<td>4.8 ± 0.2*</td>
<td>5.6 ± 0.3*</td>
<td>6.2 ± 0.5*</td>
<td>8.2 ± 0.5*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>5</td>
<td>5.3 ± 0.3*</td>
<td>5.1 ± 0.1*</td>
<td>5.6 ± 0.2*</td>
<td>6.8 ± 0.7*</td>
<td>9.1 ± 0.4*</td>
</tr>
</tbody>
</table>

Note. Values represent means ± SE; each value represents the mean for four to six different animals. Tissues were exposed for the designated time periods to 0 or 5 mM cephaloridine. No difference (p = 0.9909) was determined for total LDH (U/g tissue) between normoglycemic and diabetic group; values were 40726 ± 2514 and 40755 ± 977, respectively. Groups with different superscripts are statistically different (p < 0.05).

Numerous physiological and biochemical changes occur in the kidney of diabetic animals and humans. Streptozotocin diabetic animals rapidly develop an increase in renal blood flow and glomerular filtration rate (Jensen et al., 1981; Evan et al., 1984). The increased glomerular filtration rate would be expected to enhance renal excretion of a compound excreted primarily by filtration with minimal lumenal reabsorption. Conversely, the potential for toxicity could be enhanced if the development of toxicity was dependent on lumenal exposure to an individual toxicant. Previously published studies (Valentovic et al., 1996) had reported that renal cephaloridine levels were lower in the diabetic relative to normoglycemic rat and that cephaloridine was more rapidly removed from the kidney in diabetic compared to normoglycemic rats following a single (intraperitoneal) injection. Two potential explanations for this observation were either decreased cephaloridine uptake into the kidney or enhanced renal excretion. Diminished renal uptake did not seem to be the mechanism as plasma cephaloridine levels were similar between normoglycemic and diabetic animals. However, urinary cephaloridine excretion was higher in diabetic compared to normoglycemic rats. These studies also showed that renal cephaloridine levels that were toxic in the normoglycemic animals were not toxic in the diabetic group. Further studies presented in the current paper confirm that cephaloridine accumulation in renal slices was lower at minimally toxic doses but concentrations that induce LDH leakage in the normoglycemic group were not capable of increasing LDH leakage in the diabetic group. These findings also support the notion that toxicity can be reduced independent of renal physiological changes associated with diabetes mellitus.

Reduced renal slice accumulation was a possible explanation for the inability of cephaloridine to directly induce LDH leakage in vitro in the diabetic group. The accumulation studies indicated that diabetic tissue had lower cephaloridine levels at sublethal drug concentrations. However, cephaloridine accumulation in renal slices was comparable between normoglycemic and diabetic tissue when the cephaloridine level was elevated to 4–5 mM, a concentration sufficient to induce LDH leakage in the normoglycemic but not diabetic tissue.

TABLE 2
Effect of Cephaloridine on Total Glutathione Level in Renal Slices

<table>
<thead>
<tr>
<th>Group</th>
<th>Cephaloridine (mM)</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycemic</td>
<td>0</td>
<td>1.03 ± 0.06</td>
<td>0.84 ± 0.09</td>
<td>0.78 ± 0.06</td>
<td>0.72 ± 0.08</td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>5</td>
<td>0.78 ± 0.12</td>
<td>0.63 ± 0.14</td>
<td>0.59 ± 0.06*</td>
<td>0.39 ± 0.05*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0</td>
<td>1.26 ± 0.06</td>
<td>1.19 ± 0.08</td>
<td>1.15 ± 0.19</td>
<td>1.16 ± 0.09</td>
</tr>
<tr>
<td>Diabetic</td>
<td>5</td>
<td>1.19 ± 0.06</td>
<td>1.00 ± 0.05</td>
<td>0.96 ± 0.05</td>
<td>0.85 ± 0.06*</td>
</tr>
</tbody>
</table>

Note. Values represent means ± SE with slices from four to five different animals/groups.
* Different from respective vehicle treated tissue.
against peroxidative damage. It has yet to be resolved baseline values for glutathione. Based on these findings, it renal cortical slices from diabetic rats do not have higher compared to normoglycemic tissue. These results indicated that the diabetic kidney to handle peroxidative damage. Measurement is possible that the attenuation in renal cephaloridine damage was more resistant to depletion of glutathione when com-exposure to cephaloridine indicated that the diabetic tissue of total glutathione levels in renal slices following direct accumulation was only part of the mechanism for reduced in vitro toxicity and that other factors must be involved. The diabetic condition is associated with numerous intra-cellular changes that may influence the response of an individual cell to a toxin. The diabetic kidney has higher rates of RNA synthesis and an increased uracil ribonucleotide pool (Cortes et al., 1987). Other changes that occur in the kidney include altered expression of sodium–glucose cotransporters within the proximal tubule (Dominguez et al., 1994) and enhanced calcium–magnesium ATPase activity (Levy et al., 1986).

Very little information is known regarding the ability of the diabetic kidney to handle oxidative stress. Measurement of total glutathione levels in renal slices following direct exposure to cephaloridine indicated that the diabetic tissue was more resistant to depletion of glutathione when compared to normoglycemic tissue. These results indicated that renal cortical slices from diabetic rats do not have higher baseline values for glutathione. Based on these findings, it is possible that the attenuation in renal cephaloridine damage in diabetes may be due to an enhanced ability to protect against peroxidative damage. It has yet to be resolved whether glutathione reductase is enhanced in the streptozoto-cin model. Further studies also need to address whether the level of NADPH is higher in diabetes and what is the role of NADPH in maintaining glutathione in its reduced state. Studies conducted by other investigators using a different strain of rodent and another model of chemical induced diabetes have examined the capacity of the diabetic kidney to handle peroxidative damage. Asayama and associates (1989) reported that cytochrome c and glutathione peroxidase activity are elevated in whole kidney homogenates from Sprague–Dawley rats 14 days after streptozotocin injection. Studies conducted with alloxan-mediated diabetic Sprague–Dawley rats showed that renal tissue was more resistant to peroxidative damage by a ferrous/ascorbate free radical generating system (Parinandi et al., 1990).

In summary, cephaloridine in vitro toxicity was reduced in renal slices from diabetic Fischer 344 rats relative to normoglycemic tissue. Renal slice accumulation of cephaloridine was lower in the diabetic group at cephaloridine concentrations that produced minimal toxicity. No difference in renal slice accumulation was apparent when the cephalori-dine concentration was elevated to 4–5 mM, a concentration sufficient to induce LDH leakage only in the normoglycemic group. Total glutathione levels were more rapidly diminished in the normoglycemic tissue when compared to the diabetic group. These findings support the conclusion that diminished renal accumulation is only part of the mechanism for reduced toxicity and that an unidentified cellular difference is also part of the mechanism for reduced toxicity in streptozotocin diabetic rats.

REFERENCES


