Effects of Hydration on Gentamicin Excretion and Renal Accumulation in Furosemide-Treated Rats

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The effect of furosemide on gentamicin excretion and tissue accumulation was studied with clearance techniques in anesthetized rats, at two different infusion rates of saline or Ringer solution. Gentamicin (20 mg/kg) was administered by constant intravenous infusion over a period of 3 h. With the low fluid infusion rate, furosemide (25 mg/kg intravenously) caused severe reduction in glomerular filtration rate and diminished urinary output of gentamicin. Serum and renal tissue levels of the antibiotic were significantly elevated. High fluid infusion prevented the decline of the glomerular filtration rate, with near normalization of all measurements. A fluid deficit incurred by furosemide was noted at both the low and high infusion rates. Complete correction of this fluid deficit by continuous adjustment of the infusion rate fully restored normal renal handling of gentamicin. These results suggest that furosemide had no direct effect on renal excretion of gentamicin. In comparison, renal handling of gentamicin in rats did not respond to changes in the rate of fluid infusion in the absence of furosemide therapy. It appears that gentamicin excretion and gentamicin accumulation in the renal cortex in furosemide-treated rats, in contrast with those in untreated rats, are influenced significantly by the rate of fluid infusion. Fluid administration sufficient to maintain the glomerular filtration rate was found to be necessary for appropriate gentamicin elimination, with consequent reduction in serum and renal tissue levels of the drug.

Concentrations of gentamicin in the renal cortex and medulla were not altered by hydration in dogs (11). The present work was undertaken to ascertain whether potent loop diuretics such as furosemide have a "washout" effect and prevent gentamicin from depositing in the renal parenchyma. Since furosemide could cause renal dysfunction by severe depletion of body fluid and electrolytes (4, 6, 9), elimination of gentamicin was evaluated during furosemide therapy. The experiments were conducted at different rates of fluid infusion in an attempt to determine possible interactions between volume repletion and furosemide diuresis.

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MATERIALS AND METHODS

Thirty-nine male Sprague-Dawley rats (body weight, 228 ± 6 g) were anesthetized by intraperitoneal injection of Inactin (Promonta, Hamburg), 100 mg/kg. After tracheostomy, the left jugular vein was cannulated for blood sampling and the right femoral vein for fluid infusion. Carotid arterial blood pressure was measured with a Statham pressure transducer. The urinary bladder was catheterized for urine collection. Upon completion of surgery, the preparations underwent different experimental procedures described below.

(i) Low fluid infusion rate. Six animals were primed with 0.4 ml of normal saline containing [3H]inulin (0.8 μCi/ml) and gentamicin (1.115 mg/ml, including 5% [14C]gentamicin as [14C]gentamicin sulfate [lot no. 5883-118]; specific activity, 0.789 mCi/g; bioactivity, 628 mg/g). An infusion of the same solution at 1.2 ml/h immediately followed and was continued for 3 h. Collection of blood and urine samples began 1 h after priming dose and consisted of four consecutive clearance periods of 30 min each. At the end of each experiment, the kidneys were excised and divided into cortex and medulla for tissue analysis of gentamicin.

(ii) High fluid infusion rate. Saline (four rats) or Ringer solution (two rats), at a rate of 6.3 ml/h, was infused for 1 h preceding the administration of inulin and gentamicin as described in (i) and continued for 3 h thereafter.

(iii) Low fluid infusion rate plus furosemide. A 4-h infusion of Ringer solution, 1.2 ml/h, containing furosemide (priming dose, 5 mg/kg; sustaining dose, 5 mg/kg per h), was started 1 h before the administration of inulin as in (i) in each of six rats with and without gentamicin.

(iv) High fluid infusion rate plus furosemide.
Procedures resembled (iii) except that furosemide was delivered in 6.3 ml of Ringer solution per h.

(v) High fluid infusion rate plus furosemide and complete volume replacement. Similar to (iv) except that negative fluid balance was prevented by continuously adjusting the infusion rate of Ringer solution. All experiments except (i) consisted of six clearance periods of 20 min each.

\(^{[3]}\)H]inulin and \(^{[4]}\)Cgentamicin activities in serum, urine, or renal tissues were measured in a liquid scintillation counter with external standardization. The channels ratio method was used to account for quenching. The method provided by Isolab Inc. (Akron, Ohio) was followed in preparation of renal tissues for radioassay using “Unisol + Complement.” Preliminary studies had shown good agreement in the gentamicin values obtained with bioassay and radioassay techniques.

Student’s \(t\) test was employed in analyzing relevant parameters.

RESULTS

Influences of fluid infusion rate on renal handling of gentamicin in the absence of furosemide. A fivefold increase in infusion rate, while attended by marked rises in urine flow, resulted in only slight differences in glomerular filtration rate (GFR), as measured by inulin clearances (Table 1). With regard to steady-state serum levels of gentamicin and urinary excretion of gentamicin, no significant differences were noted between the low and high infusion groups. In addition, the drug contents in the renal cortex and medulla were similar in the two groups despite a great discrepancy in fluid infusion.

Influences of fluid infusion rate on renal handling of gentamicin in furosemide-treated rats. A brisk diuretic response to furosemide occurred within 2 h of treatment. At the end of a 4-h administration, furosemide produced fluid losses that were similar in magnitude in both the low and high infusion groups (Table 2). This was due to the fact that the latter also excreted more urine while receiving more fluid than the former. In the absence of gentamicin, the mean GFRs (in milliliters per minute per 100 g of body weight) were 0.34 ± 0.05 \((n = 4)\) for the low infusion group and 0.55 ± 0.04 \((n = 5)\) for the high infusion group, respectively, during furosemide therapy. Similar values for GFR were observed in corresponding groups that received a constant infusion of gentamicin (Table 2). Thus, under the present experimental conditions, gentamicin did not exert adverse effects on kidney function in the presence of furosemide.

The steady-state serum levels of gentamicin were markedly elevated in the low infusion group (Table 2). In addition, a striking rise in gentamicin concentration in the renal cortex and medulla was found in spite of a reduced filtered
Table 2. Renal handling of gentamicin in furosemide-treated rats

<table>
<thead>
<tr>
<th>Infusion rate</th>
<th>No. of rats</th>
<th>V (ml/min per 100 g, ×10⁻³)</th>
<th>C₅₀ (ml/min per 100 g)</th>
<th>S₀ (μg/ml)</th>
<th>L₀ (μg/ml)</th>
<th>U₀ (μg/ml)</th>
<th>U₀V (μg/min)</th>
<th>Drug concn in tissue at end of treatment (μg/g)</th>
<th>Total fluid deficit (% body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>6</td>
<td>0.90 ± 0.13</td>
<td>0.30 ± 0.03</td>
<td>11.2 ± 0.80</td>
<td>3.15 ± 0.39</td>
<td>333 ± 32</td>
<td>5.0 ± 0.5</td>
<td>338 ± 35</td>
<td>132 ± 16</td>
</tr>
<tr>
<td>High</td>
<td>6</td>
<td>4.72 ± 0.20</td>
<td>0.66 ± 0.03</td>
<td>9.46 ± 0.21</td>
<td>6.18 ± 0.26</td>
<td>110 ± 1.7</td>
<td>10.2 ± 0.3</td>
<td>144 ± 20</td>
<td>60 ± 8</td>
</tr>
<tr>
<td>High + vol replacement</td>
<td>6</td>
<td>12.64 ± 0.54</td>
<td>0.78 ± 0.06</td>
<td>7.81 ± 0.47</td>
<td>5.83 ± 0.30</td>
<td>43.5 ± 5.8</td>
<td>11.7 ± 0.5</td>
<td>135 ± 9</td>
<td>29 ± 2</td>
</tr>
</tbody>
</table>

*Furosemide: priming dose, 5 mg/kg; sustaining dose, 5 mg/kg per h; total dose given, 25 mg/kg, intravenously. Refer to Table 1 for gentamicin treatment and abbreviations.

b P < 0.05, compared with the low-infusion-rate group.

c P < 0.05, compared with the low-infusion-rate and high-infusion-rate groups.

The present data demonstrated that in the absence of furosemide, gentamicin levels in the serum and renal tissues are relatively unaffected by changes in fluid intake. Slight increases in CFR during high fluid infusions did not augment urinary excretion of the drug appreciably. Our findings were consistent with a recent report that the state of hydration in the renal cells was highly susceptible to changes in fluid intake. In contrast, however, the elevated accumulation levels in the kidneys of rats not subjected to changes in fluid intake were consistent with the drug being excreted.

Accurate replacement of fluid losses further improved CFR as well as gentamicin utilization in the urine when compared with high fluid intake. This increase in CFR may be accounted for by the associated steady-state serum levels of gentamicin. In the absence of furosemide, the high urine flow, and the associated steady-state serum levels of gentamicin (11), were different from those obtained in the absence of extrarenal therapy (Table 1). Coincidentally, there was an appreciable reduction in the tubular content of gentamicin, while the cortical gentamicin remained in the normal range.

DISCUSSION

As in dogs (1), CFR in rats would not be affected by furosemide if fluid deficit were not suppressed. Under conditions where fluid deficit was not suppressed, the fall in CFR was not accounted for by extrarenal factors. Nonetheless, under conditions where fluid deficit restored to normal levels, the elevated accumulation levels were consistent with the drug being excreted.

In conclusion, these results demonstrate that the use of fluid levels at high rates effectively lowered inulin levels and the associated steady-state serum levels of gentamicin. Therefore, the fall in CFR could be attributed to an increase in the use of fluid levels at high rates.
intratubular pressure during furosemide diuresis and an accompanying decrease in effective filtration pressure.

Clearance measurements (2) and studies with autoradiographic and brush border membrane-binding techniques (5; R. Jerrald and F. J. Silverblatt, Program Abstr. Internat. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., Abstr. no. 349, 1977) have provided evidence that gentamicin enters the renal epithelial cells via luminal membrane after filtration. The resulting cortical accumulation has been linked to gentamicin nephrotoxicity but without conclusive evidence (7, 8, 10). Assuming that zero plasma protein binding of gentamicin, as previously reported (3), occurred under the conditions of our experiments, the filtered load of gentamicin was reduced in the furosemide-treated rats receiving low fluid infusion (Table 2). Nevertheless, the cortical uptake of the drug in these animals was higher than in all other groups, suggesting enhanced reabsorption in association with diminished GFR.

Accurate correction of fluid deficit during furosemide treatment ensures complete normalization of urinary output of gentamicin by restoration of GFR. In addition, with or without full volume repletion, cortical uptake of gentamicin was normal in the presence of furosemide if fluid intake was high. Therefore, it is clear that furosemide itself has no direct influence on the urinary excretion and cortical accumulation of gentamicin. Reduced medullary concentration when urine flow is inordinately high seems to demonstrate decreased passive diffusion of the drug in the distal nephron. Since gentamicin is highly hydrophilic, passive movement in the medullary region must be limited and is undetectable in most circumstances (Table 1).

The excretion and tissue levels of gentamicin seem most affected by changes of filtration rate. The filtration rate in turn depends on rate of fluid replacement, especially during acute volume contraction as a result of concurrent use of potent natriuretic agents such as furosemide. It is concluded that furosemide, in the absence of volume repletion adequate to maintain renal function, may cause accumulation of aminoglycosides, and that furosemide does not appear to have a direct effect on renal handling of gentamicin.

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LITERATURE CITED