Influence of Hydrocortisone Succinate on Intrarenal Accumulation of Gentamicin in Endotoxemic Rats

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Gentamicin is a commonly used antibiotic in the treatment of gram-negative infections including septicemia and pyelonephritis. Bacterial endotoxin is liberated during antibiotic therapy and may lead to endotoxic shock. Steroids such as hydrocortisone are generally recommended in the treatment of endotoxic shock. There are very limited data on the influence of hydrocortisone on the pharmacology of antibiotics, especially aminoglycosides, which are nephrotoxic. We studied the influence of both Escherichia coli endotoxin and hydrocortisone succinate on the renal uptake of gentamicin in rats. Animals were injected intravenously with endotoxin (0.25 mg/kg) and/or hydrocortisone (25 mg/kg) plus gentamicin (10 mg/kg). Gentamicin levels in the serum and renal parenchyma as well as renal function and histology were evaluated. Both endotoxin and hydrocortisone given alone increased the concentration of gentamicin in the renal cortex (P < 0.05). Normal values in serum were observed in all groups at most time intervals. When administered together, endotoxin and hydrocortisone did not potentiate each other. The combination of endotoxin and hydrocortisone gave significantly higher levels of gentamicin than endotoxin or hydrocortisone alone when endotoxin was injected 3 h before hydrocortisone (P < 0.05). Blood pressure and cardiac frequency were normal when gentamicin was given. Endotoxin alone slightly decreased the glomerular filtration rate, and hydrocortisone alone slightly modified renal plasma flow. The combination of both drugs did not significantly affect renal function. No histological lesion was noted on light microscopy in animals receiving endotoxin. Competitive or synergistic activity of endotoxin, gentamicin, and hydrocortisone at the cellular level, especially on membranes or lysosomes, might explain in part our observation on the renal uptake of gentamicin. By increasing the total amount of drug within the kidney, endotoxin and hydrocortisone might increase the risk of nephrotoxicity associated with aminoglycosides.

Previous studies done in our laboratory revealed that the intrarenal distribution of gentamicin is disturbed in experimental pyelonephritis owing to Escherichia coli (7). Pyelonephritic kidneys were also shown to be more susceptible to the nephrotoxic potential of gentamicin than normal kidneys (3). The mechanism by which infection of the kidney influences the intrarenal pharmacology and the nephrotoxic potential of gentamicin is not known. Endotoxin is liberated during antibiotic therapy (29), and it may disturb the intrarenal pharmacokinetics of gentamicin (4). In fact, a greater accumulation of drug is observed in the renal cortex and medulla of endotoxin-treated animals than in the kidneys of normal animals (4). How endotoxin interacts with aminoglycosides at the tubular level is unknown, but it increases the uptake of drugs in both the proximal and distal tubules (5). Endotoxin has been shown to disturb the hemodynamic status of the host (14), activate immunological and inflammatory processes (8), induce vasoconstriction (32), and act directly on organelles to induce cell injury (24). Whether endotoxin modifies the pharmacokinetics and renal handling of drugs by acting directly on kidney cells or indirectly through one or several mechanisms remains to be elucidated.

Of the many substances used to control several of the deleterious effects of endotoxin, especially shock, steroids are the only drugs generally recommended for clinical use. Glucocorticosteroids are anti-inflammatory drugs which have been shown in both laboratory studies (23) and clinical investigations (27) to improve circulatory status and survival. The purpose of the present study was to evaluate the influence of hydrocortisone given with small doses of endotoxin on the intrarenal uptake of a potentially nephrotoxic aminoglycoside, gentamicin.

( Portions of this work were presented at the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, Minn., October 1985, abstr. 707.)

MATERIALS AND METHODS

Experimental model: distribution studies. Female Sprague-Dawley rats weighing 175 to 200 g were used for all experiments. A total of 151 animals were anesthetized by a single intraperitoneal injection of sodium pentobarbital (45 mg/kg), followed 2 h later by a second dose of 20 mg/kg. Catheters were inserted into the right jugular vein for infusion of solutions. E. coli O127:B8 endotoxin (0.25 mg/kg; Difco Laboratories, Detroit, Mich.) or normal saline was infused intravenously over a period of 15 min at the beginning of the experiment. This low dosing of endotoxin was chosen to avoid major physiological disturbances in the Sprague-Dawley rats. Hydrocortisone succinate (25 mg/kg; Solu-cortef; The Upjohn Co., St-Laurent, Quebec, Canada) was given intravenously over a period of 1 min either 15 min or 2 h and 58 min after the beginning of infusion of endotoxin. This allowed two types of observation: an early protection against reactions such as inflammation induced by endotoxin, and a situation similar to clinical practice with endotoxin circulating freely for several hours before the treatment. At 3 h after endotoxin, an intravenous bolus of gentamicin (10 mg/kg; Schering Canada, Pointe-Claire, Quebec, Canada) was given. This dose resulted in levels in serum close to values found in humans. Normal saline was infused at a rate of 1.15 ml min⁻¹/kg⁻¹ for 1 h.
ml/h throughout the experiments. The different treatment regimens are presented in Table 1.

Blood was taken by cardiac puncture and centrifuged 0.5, 1, 2, and 4 h after injection of gentamicin. Kidneys were removed at the same time, separated into cortex, medulla, and papilla, and homogenized in 0.1 M phosphate buffer (pH 7.4). Urine was also collected. The concentrations of drug were determined by a standard disk biological assay (6). All assays were done in triplicate on tryptic soy agar (Difco) with *Bacillus subtilis* as the test organism. Standard curves for the antibiotic assays were prepared with serum for serum, with physiological saline for urine, and with cortex, medulla, and papilla homogenates for the renal tissue. The levels of recovery of gentamicin after known amounts of drug-free homogenates were added was 98 ± 1.6%. Endotoxin and hydrocortisone did not affect bacterial growth and did not interfere with gentamicin during the assay.

**Pharmacokinetics.** The elimination rate constant (k\(e\)), the elimination half-life (t\(1/2\), k\(e\)), the area under the curve from 0 h to infinity (AUC\(0\rightarrow\infty\)), and the volume of distribution (\(V\); k\(d\)) were determined by using model I (one compartment with bolus input and first-order output) of Pc Nonlin software (Statistical Consultants, Lexington, Ky.). The total plasma clearance was calculated as follows: dose of gentamicin/AUC\(0\rightarrow\infty\).

**Physiological studies.** Blood pressure and cardiac frequency were recorded with a model R711 physiograph (Beckman Instruments, Inc., Fullerton, Calif.) in all groups from 0 to 7 h after beginning of the experiments.

Renal function was evaluated in all groups listed in Table 1 from 1 to 2.5 hours after gentamicin injection. In these experiments, five rats of each group were anaesthetized with pentobarbital (45 mg/kg). The left carotid artery and the right jugular vein were cannulated with type PE-50 and PE-20 polyethylene tubing (Intramedic Clay Adams, Parsippany, N.J.), respectively. The former was used to sample blood, while the latter was used for infusion. Immediately after gentamicin treatment, a 1-ml bolus of 0.85% NaCl containing \(^{14}\text{C} \) inulin (0.10 mCi/100 ml; New England Nuclear Corp., Boston Mass.), \(^{3} \)H]aminohippuric acid (\(^{3} \)HPAH; 0.50 mCi/100 ml; New England Nuclear Corp.), unlabeled inulin (1 mg/ml), and unlabeled PAH (10 mg/ml) was infused at a rate of 0.28 ml/min. This bolus was immediately followed by continuous infusion of the same solution at a rate of 0.052 ml/min; 1 h later, urine was collected for three sequential 30-min periods from the right and left ureters, which were separately cannulated with type PE-10 tubing.

Blood samples were taken at the beginning and the end of each urine collection period. At the end of the last period, blood was also taken from the renal vein to measure the renal plasma flow (RPF). \(^{14}\text{C} \) inulin and \(^{3} \)HPAH were measured with a Beckman model LS 7500 liquid scintillation

counter by using a double-label program and automatic quench correction. The glomerular filtration rate was determined by inulin clearance. Tubular secretion was evaluated with PAH. RPF was calculated by using the following equation: RPF = \((\text{PAH in urine} - \text{PAH in renal venous plasma}) \times V/(\text{PAH in arterial plasma} - \text{PAH in renal venous plasma})\), where V was the urine flow (in milliliters per minute).

**Histology.** Histological observations were made by using kidneys from animals that received either no endotoxin or 0.25 mg of endotoxin per kg. The animals were sacrificed 3 h, 4 h, or 1 day after endotoxin treatment. At the time of sacrifice, a midline abdominal incision was made, and a 21-gauge butterfly needle was inserted into the aorta above the renal arteries. After perfusion of 10 ml of Krebs-Ringer solution (pH 7.4) at a rate of 7 ml/min, 100 ml of 2% glutaraldehyde (pH 7.4) was infused into the live animals. After the in vivo glutaraldehyde fixation, the kidneys were excised and placed in the same fixative for 2 h. Cubes (1 mm\(^3\)) were removed from the cortex and medulla and were left overnight in the same fixative at 4°C. After being washed with 0.1 M phosphate buffer (pH 7.4), the cubes were further fixed in 2% osmium tetroxide for 3 h at 4°C, dehydrated in ascending grades of alcohol, and embedded in type 502 araldite resin. Thick sections (1 μm) were cut with an LKB Ultratome III, stained with toluidine blue, and examined by using a blind code to identify gross lesions.

**Statistics.** Results were compared by using an analysis of variance. Comparison of group means was done by using the Duncan multiple range test with Kramer’s adjustment for unequal frequencies (21a).

**RESULTS**

**Aminoglycoside pharmacokinetics.** (i) Serum. The concentrations of gentamicin in sera are shown in Table 2. Thirty minutes after injection, a higher peak level was observed in animals treated with endotoxin alone than in normal animals (P < 0.05). Thereafter, from 1 to 4 h the mean concentrations at each time point were not significantly different from control values. On the contrary, hydrocortisone injected alone induced lower levels of gentamicin in serum at 30 min than did normal saline. When hydrocortisone was injected immediately after endotoxin (Table 2, EH + G), the levels of gentamicin were normal. High levels in blood were observed at 1 h with hydrocortisone given 3 h after endotoxin (E + HG).

The pharmacokinetic data of gentamicin for all groups are shown in Table 3. Compared with normal saline, a slightly lower V associated with a slightly higher AUC was observed in rats given endotoxin alone. On the contrary, after hydrocortisone alone, slightly higher Vs associated with lower AUC were observed. Endotoxin plus hydrocortisone resulted in normal AUC when given concurrently (Table 3,

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**TABLE 1.** Different treatment regimens

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of administration</th>
<th>0 h</th>
<th>15 min</th>
<th>2 h 58 min</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>Normal saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E + G</td>
<td>Endotoxin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H + G</td>
<td>Hydrocortisone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EH + G</td>
<td>Endotoxin</td>
<td>Hydrocortisone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>Endotoxin</td>
<td>Hydrocortisone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E + HG</td>
<td>Endotoxin</td>
<td>Hydrocortisone</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The respective doses of endotoxin, hydrocortisone, and gentamicin were 0.25, 25, and 10 mg/kg.

* G, Gentamicin; E, endotoxin; H, hydrocortisone.
TABLE 2. Concentrations in serum of gentamicin in rats treated with endotoxin, hydrocortisone, and gentamicin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc of gentamicin (µg/ml) in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 h</td>
</tr>
<tr>
<td>G</td>
<td>9.8(0.2)b</td>
</tr>
<tr>
<td>E + G</td>
<td>14.1(2.9)a</td>
</tr>
<tr>
<td>H + G</td>
<td>6.7(0.8)c</td>
</tr>
<tr>
<td>EH + G</td>
<td>7.3(0.5)bc</td>
</tr>
<tr>
<td>HG</td>
<td>6.7(0.7)c</td>
</tr>
<tr>
<td>E + HG</td>
<td>7.3(0.4)bc</td>
</tr>
</tbody>
</table>

* G, Gentamicin; E, endotoxin; H, hydrocortisone. See Table 1 for treatment regimens.

TABLE 3. AUC, elimination half-life, V, and total clearance of gentamicin in rats treated with endotoxin, hydrocortisone, and gentamicin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC&lt;sub&gt;0→∞&lt;/sub&gt; (µg · h/ml)</th>
<th>Elimination half-life (h)</th>
<th>V (ml/kg)</th>
<th>Total clearance (ml/min per kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>17.8(1.1)bc</td>
<td>0.81(0.10)a</td>
<td>659 (57)abc</td>
<td>9.4 (0.6)bc</td>
</tr>
<tr>
<td>E + G</td>
<td>21.0(2.2)c</td>
<td>0.60(0.16)</td>
<td>411 (96)a</td>
<td>8.4 (0.9)c</td>
</tr>
<tr>
<td>H + G</td>
<td>12.0(1.0)ab</td>
<td>0.73(0.13)</td>
<td>880 (116)bcd</td>
<td>13.9 (1.2)ab</td>
</tr>
<tr>
<td>EH + G</td>
<td>16.0(2.6)abc</td>
<td>1.20(0.32)</td>
<td>1,072 (178)cd</td>
<td>10.4 (1.7)abc</td>
</tr>
<tr>
<td>HG</td>
<td>11.5(1.0)a</td>
<td>0.77(0.13)</td>
<td>970 (123)cd</td>
<td>14.5 (1.3)a</td>
</tr>
<tr>
<td>E + HG</td>
<td>26.8(2.7)d</td>
<td>0.92(1.27)</td>
<td>496 (138)ab</td>
<td>6.2 (0.6)d</td>
</tr>
</tbody>
</table>

* G, Gentamicin; E, endotoxin; H, hydrocortisone. See Table 1 for treatment regimens. Values obtained at 0.5 h in the E + HG group were not used for the calculation of these parameters since they were lower than those at 1.0 h.

* Numbers are means for six to seven animals. Values within parentheses are standard error of the mean.

* For each variable, means followed by a common letter are not significantly different.

(iii) Urine. The diuresis and percentage of gentamicin recovered in urine are shown in Table 5. Less antibiotic was recovered in urine of animals treated with endotoxin alone than in urine of normal rats 2 h after injection. On the contrary, high excretion of gentamicin was observed during the first hour after hydrocortisone alone. The administration of both endotoxin and hydrocortisone resulted in normal or low urinary gentamicin excretion.

Physiological studies. (i) Blood pressure and pulse rate. After endotoxin treatment, systolic pressure was unchanged. The diastolic pressure decreased from 80 mm Hg in the normal rats to 61 mm Hg in the endotoxemic animals at 2 h, but was normal at 3 h. The pulse rate was more rapid in the endotoxin-treated animals between 2 and 3 h after injection of endotoxin but came back to normal thereafter. Hydrocortisone alone did not alter blood pressure or pulse rate. Combined with endotoxin, hydrocortisone did not neutralize the endotoxin effects. A fall from 110 to 83 mm Hg in systolic pressure followed by a return to normal was observed from 1 to 2 h postendotoxin.

FIG. 1. Concentrations (micrograms per gram) of gentamicin in the renal cortex from 0.5 to 4.0 h after injection of animals with endotoxin and hydrocortisone. Abbreviations: G, gentamicin; E + G, endotoxin plus gentamicin 3 h later; H + G, hydrocortisone plus gentamicin 3 h later; EH + G, endotoxin and hydrocortisone at the same time plus gentamicin 3 h later; HG, hydrocortisone and gentamicin at the same time; E + HG, endotoxin plus hydrocortisone and gentamicin 3 h later. Values at each time interval are means for six to seven rats.
TABLE 5. Diuresis and percentage of the dose recovered in urine at different time intervals in animals treated with endotoxin, hydrocortisone, and gentamicin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-1 h</th>
<th>1-2 h</th>
<th>3-4 h</th>
<th>0-1 h</th>
<th>1-2 h</th>
<th>3-4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>0.77 (0.30)</td>
<td>0.73 (0.13)ab</td>
<td>0.56 (0.13)a</td>
<td>31.9 (3.6)a</td>
<td>15.8 (1.5)b</td>
<td>4.0 (0.9)b</td>
</tr>
<tr>
<td>E + G</td>
<td>0.50 (0.04)a</td>
<td>0.32 (0.04)a</td>
<td>0.43 (0.10)a</td>
<td>24.8 (6.4)a</td>
<td>5.8 (0.9)a</td>
<td>2.4 (0.8)ab</td>
</tr>
<tr>
<td>H + G</td>
<td>0.96 (0.17)a</td>
<td>0.74 (0.17)a</td>
<td>0.71 (0.07)a</td>
<td>47.0 (0.43)b</td>
<td>10.6 (1.1)ab</td>
<td>0.9 (0.2)a</td>
</tr>
<tr>
<td>EH + G</td>
<td>0.74 (0.08)a</td>
<td>0.98 (0.15)ab</td>
<td>0.68 (0.13)a</td>
<td>24.9 (5.3)a</td>
<td>12.3 (1.7)ab</td>
<td>1.5 (0.5)a</td>
</tr>
<tr>
<td>HG</td>
<td>0.64 (0.09)a</td>
<td>0.56 (0.12)a</td>
<td>0.59 (0.10)a</td>
<td>46.8 (2.7)b</td>
<td>14.7 (4.4)b</td>
<td>2.6 (0.5)ab</td>
</tr>
<tr>
<td>E + HG</td>
<td>1.00 (0.18)a</td>
<td>0.46 (0.05)ab</td>
<td>0.69 (0.31)a</td>
<td>29.3 (5.1)a</td>
<td>8.0 (0.7)a</td>
<td>2.3 (0.8)ab</td>
</tr>
</tbody>
</table>

* G, Gentamicin; E, endotoxin; H, hydrocortisone. See Table 1 for treatment regimens.
* Values are means followed by standard error of the mean. At each time, means followed by a common letter are not significantly different.

(ii) Renal function. Table 6 shows the data for inulin clearance, PAH secretion, and RPF for all groups. A slight decrease in glomerular filtration rate was observed after endotoxin alone, while hydrocortisone alone (H + G) resulted in increased glomerular filtration rate. Hydrocortisone alone significantly decreased PAH secretion when given a few minutes before gentamicin (HG). However, renal function including tubular secretion was normal in animals receiving both endotoxin and hydrocortisone.

Histology. No histological lesions were observed when light microscopy was used to examine animals that received endotoxin. The brush border was intact, and lysosomes were of normal size.

DISCUSSION

There are very limited data on the influence of endotoxin or corticosteroids on the pharmacology, efficacy, and toxicity of antibiotics. Endotoxin, an important constituent of the cell wall of gram-negative microorganisms, has been considered as the major offender responsible for the many complications associated with severe infections including septic shock (10). After endotoxin, vasoactive mediators such as histamines, serotonin, bradykinins, catecholamines, and prostaglandins are released from blood cells and tissues and are responsible for vasoconstriction-vasodilatation cycles, intravascular pooling, decreased tissue perfusion, decreased venous return and cardiac output, systemic hypotension, lung, kidney, or heart failure, and death (17).

While an important goal of treatment is to eliminate the causative microorganism by the use of antibiotics such as aminoglycosides, therapy is also directed toward correcting the hemodynamic abnormalities associated with septic shock. Appropriate measures include the administration of glucocorticosteroids such as hydrocortisone which have been shown in both laboratory studies and clinical investigations to improve circulatory status and survival (23, 27). They interfere with the release of histamine (1) and prostaglandins (9), which play a role in inflammation (22). They block the vasoconstriction effects of endotoxin (21) and produce renal vasodilatation with ensuing improvement in renal parenchymal perfusion (31).

In previous experiments (4, 5), we have shown that endotoxin disturbs the intrarenal pharmacokinetics of aminoglycosides which are nephrotoxic drugs. In the present study, we evaluated the influence of hydrocortisone on the intrarenal uptake of gentamicin in endotoxemic animals. Our major finding was a significantly greater uptake of gentamicin in the renal parenchyma of animals treated with endotoxin or hydrocortisone or both than in normal animals.

The hemodynamic status of the animals was minimally affected by endotoxin. Both pulse rate and blood pressure were normal when gentamicin was given. RPF was not significantly disturbed. But even at the low dose given, endotoxin alone slightly decreased V and the glomerular filtration rate.

FIG. 2. Total amount of gentamicin (micrograms) 2 h after injection recovered in the whole kidneys of animals treated with endotoxin and hydrocortisone. Abbreviations: G, gentamicin; E + G, endotoxin plus gentamicin 3 h later; H + G, hydrocortisone plus gentamicin 3 h later; EH + G, endotoxin and hydrocortisone at the same time plus gentamicin 3 h later; HG, hydrocortisone and gentamicin at the same time; E + HG, endotoxin plus hydrocortisone and gentamicin 3 h later. Values are means for six to seven animals ± standard error of the mean (bars). Means with a common letter are not significantly different at the 5% level, as shown by the Duncan multiple range test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glomerular filtration rate (ml/min)</th>
<th>PAH secretion (μmol/min)</th>
<th>RPF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>0.365 (0.042)a</td>
<td>0.224 (0.089)b</td>
<td>2.59 (0.504)a</td>
</tr>
<tr>
<td>E + G</td>
<td>0.287 (0.064)a</td>
<td>0.252 (0.043)b</td>
<td>2.37 (0.895)a</td>
</tr>
<tr>
<td>H + G</td>
<td>0.504 (0.035)b</td>
<td>0.285 (0.049)b</td>
<td>3.23 (0.755)a</td>
</tr>
<tr>
<td>EH + G</td>
<td>0.388 (0.017)ab</td>
<td>0.351 (0.023)b</td>
<td>3.88 (0.156)a</td>
</tr>
<tr>
<td>HG</td>
<td>0.394 (0.40a)b</td>
<td>0.079 (0.04)ab</td>
<td>2.03 (0.32)a</td>
</tr>
<tr>
<td>E + HG</td>
<td>0.350 (0.023)a</td>
<td>0.272 (0.042)b</td>
<td>3.54 (0.403)a</td>
</tr>
</tbody>
</table>

* All values in the table are means for the right kidneys. n = 5 for glomerular filtration rate and PAH secretion, and n = 4 for RPF. Values in parentheses are standard error of the mean; means followed by a common letter are not significantly different at the 5% level, as shown by the Duncan multiple range test.
* G, Gentamicin; E, endotoxin; H, hydrocortisone.
* Values of glomerular filtration rate are for 100 g of body weight.
filtration rate, possibly through the release of vasoactive agents with subsequent intravascular pooling. This was most likely responsible for the increased levels in serum observed 30 min after gentamicin administration, the slight increase in AUC, and the decrease in urinary recovery of drug. The high antibiotic levels in serum could have resulted in better availability of gentamicin for transport in the kidneys, leading to the high intracortical concentrations of drug observed in the animals treated with endotoxin alone.

Our data for gentamicin are identical to those of Wilson et al. (34), who noticed elevated gentamicin concentrations in serum and a decrease in the half-life and in the apparent V of gentamicin in horses treated with E. coli O55:B5 endotoxin.

However, levels in serum cannot be the sole explanation for the disturbed intrarenal uptake of gentamicin that we observed since hydrocortisone given alone or concurrently with endotoxin (EH + G) also resulted in high intrarenal accumulation of gentamicin despite low levels in serum.

Hydrocortisone alone resulted in lower levels of gentamicin in serum, especially during the first hour, low serum AUCs, and higher V's and glomerular filtration rates than in normal animals. This was associated with an increased urinary excretion of gentamicin. Hydrocortisone might have interfered with the normal release of vasoactive hormones leading to the slight modifications observed. Diuresis was normal.

However, tubular reabsorption seemed to be affected since high renal uptake of gentamicin was seen in animals receiving hydrocortisone alone. More so, when both endotoxin and hydrocortisone were given, high renal uptake was observed despite normal renal function, suggesting that besides the physiological changes observed in our experiment, endotoxin and hydrocortisone acted directly on the tubular transport of gentamicin.

After filtration through the glomeruli, gentamicin is reabsorbed in the proximal tubular cells by pinocytosis (30). Both endotoxin and aminoglycosides seem to bind to receptor sites on membrane phospholipids (2). The phospholipid receptor seems to be an anionic binding site that is shared by polypeptides, amino acids, aminoglycosides, and cationic proteins that are reabsorbed (M. Sastrasinh, T. C. Knauuss, J. M. Weinberg, and H. D. Humes, Kidney Int. 19:213, 1981). Since endotoxin has been shown to increase the negative charge on liposomes formed from different phospholipids (25), we hypothesize that the binding between positively charged gentamicin (a polycationic drug) and the negatively charged phospholipids was enhanced by endotoxin, thus leading to the increased incorporation of gentamicin that we observed within the tubular cells.

Hydrocortisone itself can influence, directly or indirectly, numerous kidney functions. Glucocorticosteroids have been shown to modify renal hemodynamics (11), acid and water excretion (18, 28), and Na⁺-K⁺ ATPase activity and tubular transport (26). The exact location of hydrocortisone receptors in the nephron is not known, but after filtration through the glomeruli, hydrocortisone would be reabsorbed by passive tubular transport without a transport maximum (12). Corticosteroids have also been found to influence cell membrane permeability (16).

In our experiment, hydrocortisone combined with endotoxin resulted in greater accumulation of gentamicin in the cortex and the whole kidney than in normal animals. The effect was maximal with hydrocortisone given shortly before gentamicin. In that group (E + HG), endotoxin could not freely on kidney cells during 3 h, and a cumulative effect of endotoxin and hydrocortisone was seen on the renal uptake of gentamicin. The pharmacokinetic data (high AUC, low V, low total clearance, low urinary excretion at 2 h) looked quite the same as those observed with the animals receiving endotoxin alone (E + G), but the kidney levels of drug were significantly higher in the E + HG group than in the E + G group, suggesting again that endotoxin or hydrocortisone or both acted directly on the tubular transport of gentamicin.

In contrast, when given concomitantly, both endotoxin and hydrocortisone did not potentiate each other, and the intrarenal uptake of gentamicin was not greater than with either drug given alone. We believe that competitive or synergistic activity of endotoxin, hydrocortisone, and gentamicin on receptor sites on tubular membranes could explain, at least in part, our observations on the renal uptake of gentamicin. Competitive activity would occur with endotoxin and hydrocortisone administered at the same time, while cumulative effects would occur with both drugs given separately.

This is consistent with the findings of Geller et al. (13), who noticed that concurrent administration of glucocorticosteroids protected a significant number of animals against endotoxin lethality. When the injection was delayed, the animals rapidly lost the ability to respond to the protective effects of the hormone, and death ensued.

After their entrance within the cells, aminoglycosides accumulate within the lysosomes (30). Lysosomal membranes and enzymes are also target organelles of endotoxin (20) and hydrocortisone (19). Loss of lysosomal membrane integrity might explain in part the rapid elimination that followed the rapid uptake of gentamicin in the kidneys of animals treated with endotoxin or hydrocortisone or both.

By increasing the total amount of gentamicin within the kidney, both endotoxin and hydrocortisone might increase the risk of nephrotoxicity associated with aminoglycosides. Ture and Hsu (33) and Godin and Tuchek (15) have also mentioned that under certain conditions there might even be a synergistic toxic molecular interaction between endotoxin and antibiotics.

On the basis of our observations, we suggest that careful monitoring of patients receiving steroids for either shock or other underlying disease would be warranted.

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LITERATURE CITED


