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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 [^{3}H]inulin (0.8 μCi/ml) and gentamicin (1.115 mg/ml, including 5% [^{14}C]gentamicin as [^{14}C]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 [^{14}C]gentamicin 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 1. *Renal handling of gentamicin in normal rats*[a]

Infusion rate	No. of rats	V (ml/min per 100 g, ×10^{-2})	C_{IN} (ml/min per 100 g)	S_G (μg/ml)	L_G (μg/min per 100 g)	U_G (μg/ml)	U_GV (μg/min)	Drug concn in tissue at end of treatment (μg/g)[b]	
								Cortex	Medulla
Low	6	0.34 ± 0.03	0.70 ± 0.04	8.05 ± 0.81	5.47 ± 0.37	1,623 ± 154	12.8 ± 0.8	130 ± 20	72 ± 10
High	6	2.80 ± 0.40	0.84 ± 0.02	7.50 ± 0.38	6.39 ± 0.46	209 ± 21	13.6 ± 0.9	116 ± 8	60 ± 3
P value		<0.05	<0.05	NS[c]	NS	<0.05	NS	NS	NS

[a] Priming dose for gentamicin, 446 μg per animal; sustaining dose, 22.3 μg/min per animal. The total dose administered was approximately 20 mg/kg intravenously. V, Urine flow rate, C_{IN}, inulin clearance, and L_G, filtered load of gentamicin, are expressed per 100 g of body weight; S_G, steady-state serum levels of gentamicin; U_GV, urinary excretion rate of gentamicin; U_G, urinary concentration of gentamicin. All represent mean values ± standard error during the last 2 h of a 3-h infusion of the drug.

[b] Wet tissue weight is used to express drug concentrations in the renal tissues that were obtained at the end of drug treatment.

[c] NS, Not significant.

Table 2. *Renal handling of gentamicin in furosemide-treated rats*[a]

Infusion rate	No. of rats	V (ml/min per 100 g, $\times 10^{-2}$)	C_{IN} (ml/min per 100 g)	S_G (μg/ml)	L_G (μg/min per 100 g)	U_G (μg/ml)	U_GV (μg/min)	Drug concn in tissue at end of treatment (μg/g)		Total fluid deficient (% body wt)
								Cortex	Medulla	
Low	6	0.90 ± 0.13	0.30 ± 0.03	11.2 ± 0.80	3.15 ± 0.39	333 ± 32	5.0 ± 0.5	338 ± 35	132 ± 16	1.73 ± 0.53
High	6	4.72 ± 0.20[b]	0.66 ± 0.03[b]	9.46 ± 0.21	6.18 ± 0.26[b]	110 ± 1.7[b]	10.2 ± 0.3[b]	144 ± 20[b]	60 ± 8[b]	1.49 ± 0.22
High + vol replacement	6	12.64 ± 0.54[c]	0.78 ± 0.06[b]	7.81 ± 0.47[c]	5.83 ± 0.30[b]	43.5 ± 5.8[c]	11.7 ± 0.5[c]	135 ± 9[b]	29 ± 2[c]	

[a] 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.

load (=steady-state gentamicin serum level × GFR) as compared to non-furosemide-treated animals (Table 1).

In comparison with the low infusion group, the use of fluid infusion at high rates effectively lowered drug levels in the renal tissues (Table 2). Excretion and steady-state serum levels of gentamicin were ameliorated, accompanying an increase in GFR.

Accurate replacement of fluid losses further improved GFR as well as gentamicin eliminated in the urine when compared with the high infusion group that suffered fluid deficit despite high fluid intake (Table 2). Values of these measurements and the associated steady-state serum levels of gentamicin were not different from those obtained in the absence of furosemide therapy (Table 1). Coincidental with extraordinarily high urine flow, there was an appreciable reduction in the medullary content of gentamicin, while the cortical gentamicin remained in the normal ranges.

DISCUSSION

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 infusion. Slight increases in GFR during high fluid infusion did not augment urinary excretion of the drug appreciably. Our findings were consistent with a recent report that the state of hydration in normal dogs does not influence intrarenal distribution of gentamicin (11). In contrast with untreated rats, GFR in the furosemide-treated animals was highly susceptible to changes in rate of fluid infusion. GFR was rendered less than half of normal by furosemide when fluid infusion was low and was considerably improved with increased infusion. Simultaneously, the elevated serum levels, diminished excretion, and increased accumulation of gentamicin in the kidney in association with low fluid infusion were ameliorated by high fluid infusion during furosemide.

As in dogs (1), GFR in rats would not be affected by furosemide if fluid deficit were prevented by continuous replacement for urinary losses. Nevertheless, under conditions where fluid correction was incomplete, suppression of GFR by furosemide was more marked in animals receiving low infusion than those with high infusion despite the similar net fluid deficit in both groups at the end of experiments. Therefore, fluid depletion alone did not seem to account for the fall in GFR (6). Duchin et al. (K. L. Duchin, L. N. Peterson, and T. J. Burke, Abstr. Annu. Meet. Am. Soc. Nephrol., 10th, Washington, D.C., 1977, Abstr. no. 103) recently demonstrated that the decline in GFR, barring volume changes, could be attributed to an increase in

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. Jerrauld and F. J. Silverblatt, Program Abstr. Intersci. 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

1. **Burke, T. J., R. R. Robinson, and J. R. Clapp.** 1972. Determinants of the effect of furosemide on the proximal tubule. Kidney Int. 1:12-18.
2. **Chiu, P. J. S., A. Brown, G. Miller, and J. F. Long.** 1976. Renal extraction of gentamicin in anesthetized dogs. Antimicrob. Agents Chemother. 10:277-282.
3. **Gordon, R. C., C. Regamy, and W. M. M. Kirby.** 1972. Serum protein binding of the aminoglycoside antibiotics. Antimicrob. Agents Chemother. 2:214-216.
4. **Greenblatt, D. J., D. W. Duhme, M. D. Allen, and J. Koch-Weser.** 1977. Clinical toxicity of furosemide in hospitalized patients. Am. Heart J. 94:6-13.
5. **Habermann, E.** 1977. Transmembranal and intracellular transport of pharmacologically active proteins and polypeptides. Naunyn-Schmiedeberg's Arch. Pharmacol. 297(Suppl.):11-14.
6. **Jewkes, R. F., N. Burki, and A. Guz.** 1970. Observations of renal function in patients undergoing therapeutic diuresis with furosemide. Clin. Sci. 38:439-449.
7. **Luft, F. C., and S. A. Kleit.** 1974. Renal parenchymal accumulation of aminoglycoside antibiotics in rats. J. Infect. Dis. 130:656-659.
8. **Luft, F. C., J. Patel, M. N. Yum, and S. A. Kleit.** 1976. Nephrotoxicity of cephalosporin-gentamicin combinations in rats. Antimicrob. Agents Chemother. 9:831-839.
9. **Tilstone, W. J., P. F. Semple, D. H. Lawson, and J. A. Boyle.** 1977. Effects of furosemide on glomerular filtration rate and clearance of practolol, digoxin, cephaloridine, and gentamicin. Clin. Pharmacol. Ther. 22:389-394.
10. **Wahlig, H., A. Metallinos, W. Hameister, and R. Bergmann.** 1974. Gentmycin-Konzentrationen in Geweben und Körperflüssigkeiten von Versuchstieren. Int. J. Clin. Pharmacol. 10:212-229.
11. **Whelton, A., and W. G. Walker.** 1974. Intrarenal antibiotic distribution in health and disease. Kidney Int. 6:131-137.