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Effect of Acetazolamide on the Diuretic Action of Furosemide in Rabbits*

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=국문초록=

Acetazolamide가 Furosemide의 이뇨작용에 미치는 영향

가톨릭의대 약리학교실

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Furosemide의 이뇨작용에 대한 acetazolamide의 영향을 알아보고자 토끼를 4군으로 나누어 saline (0.5ml/kg), acetazolamide (10mg/kg), furosemide (0.5mg/kg), acetazolamide (10mg/kg) +furosemide(0.5 mg/kg)를 각각 정맥주사하였다.

1) Acetazolamide와 furosemide병합투여시 furosemide를 단독투여했을 때의 뇨량, 뇨중 전해질 배설량에 비하여 유의한 감소를 보였다.

2) Acetazolamide와 furosemide병합투여시와 furosemide 단독투여시의 뇨중 전해질과 뇨량의 fractional excretion rate를 비교하면, 병합투여시에 감소되어 나타났으며 뇨중전해질보다 뇨량에서 그 정도가 심하였다.

3) Acetazolamide와 furosemide병합투여시 뇨 pH가 furosemide 단독투여시보다 높게 나타났다.

4) 뇨중 Cl^-배설량에 대한 $Na^+ + K^+$배설량의 비는 acetazolamide + furosemide병합투여군과 furosemide 단독투여군에서 유의한 차이를 보이지 않았다.

Acetazolamide와의 병합투여로 나타나는 furosemide 이뇨작용의 감소는 뇨 pH의 증가 또는 ascending Henle's limb에서의 Cl^-재흡수 억제의 감소에 기인하는 것으로 사료된다.

Introduction

Furosemide, 4-chloro-N-(2-furyl-methyl)-5-sulfamyl anthranilic acid, is strongly bound to plasma proteins and majority of its urinary output is resulted from proximal tubular secretion, and some organic acids such as probenecid, block its secretion (Wallin et al., 1976), and its diuretic action is also reduced by probenecid(Honari et al., 1977).

It has been confirmed that the primary action of furosemide is due to the inhibition of active reabsorption of chloride ion at the luminal side of ascending Henle's loop(Burg, 1976), and its diuretic responses were altered by the change of urinary pH(Kim & Cho, 1980).

Since acetazolamide increases the alkalinity of the urine by virtue of inhibition of proximal tubular carbonic anhydrase, the urinary concentration of bicarbonate anions increases whereas urinary concentration of chloride falls. The question arises whether alteration of urinary concentration

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of anion composition derived from acetazolamide might influence the renal action of furosemide.

Authors studied the diuretic activities of furosemide when acetazolamide was administered prior to or combined with furosemide in the rabbit.

Materials and Methods

Male rabbits ranging in weight from 2 to 3 kg were anesthetized by the intraperitoneal injection of 25% of urethan (5 ml/kg). A midline incision of the skin of the neck was made and a tracheal cannula was inserted. The left femoral artery and vein were catheterized in order to permit blood sampling and intravenous infusion of saline and drugs. A midline incision of abdomen was made, and both ureters were catheterized with polyethylene tube (No. 18).

Saline, containing 0.3% inulin, was infused into left femoral vein at the rate of 0.5 ml/kg/min. Urine flow was usually stabilized within one hour following completion of surgical procedure. Experiment was started after one hour equilibration period.

Blood samples were obtained from femoral artery in heparinized centrifuge tubes at the midpoint of each collection of urine. Plasma was separated from blood cells by centrifugation at 2,500 rpm for 20 minutes. After three 10 minute collection of urine as a control, drugs were intravenously administered as described in the following schedule, and each six sample of 10 minute collection of urine and blood were obtained.

The urine volume, ionic compositions, pH, osmolality and GFR were determined for each sample.

Dose schedule was as follows:

a. Saline: 0.5 ml/kg

b. Acetazolamide(Diamox, Lederle Lab. N.Y. U.S.A.): 10 mg/kg

c. Furosemide(Lasix, Han Dok Pharm. LTD.); 0.5 mg/kg

d. Acetazolamide; 10 mg/kg+Furosemide; 0.5 mg /kg

The concentration of sodium and potassium was determined with flamephotometer(Instrumentation Lab. Model 143, U.S.A.), and chloride concentration was determined with chloridometer(Buchler-Cotlove Instrumentation, U.S.A.). Osmolarity was determined with Osmette (Precision Osmometer Inc. U.S.A.). Inulin was analyzed by method of Schreiner(1950).

Results

Fig. 1 shows the mean urine volume over 30 minutes period after administration of drugs in the rabbit. Urine flow after saline injection varied from 0.43 to 0.47 ml/min. The urine volume at 10 minute period after administration of acetazolamide was 0.75±0.07 ml/min., and that of furosemide was 2.58±0.42 ml/min, respectively. The urine volume at 10 minutes after combined administration of acetazolamide and furosemide was 0.98±0.12 ml/min., and this value was significantly lower than that of single administration of furosemide ($p<0.05$).

The electrolytes output at 10 minutes period after administration of drugs is shown in Fig. 2. Urinary output of sodium and chloride was significantly lower after combined administration of acetazolamide and furosemide compared with that of single administration of furosemide ($p<0.05$).

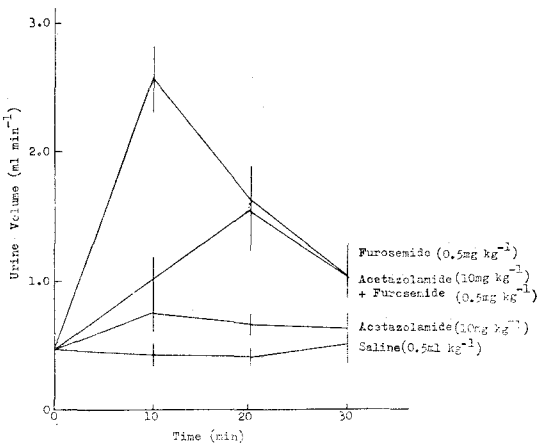

Fig. 1. Urine volume over 30 minutes after administration of drugs in the rabbit. (Mean± S.D., n=8)

Table 1. Fractional excretion rate of urinary sodium, chloride, urine flow and urinary pH over 10 minutes after administration of drugs in rabbit

Drugs (I.V.)	$\frac{U_{Na^+}\cdot V}{GFR}$ (mEq L^{-1})	$\frac{U_{Cl^-}\cdot V}{GFR}$ (mEq L^{-1})	$\frac{U\cdot V}{GFR}$	U_{pH}
Saline(8)* 0.5 ml kg^{-1}	6.89±0.13	7.32±0.14	0.04±0.02	6.84±0.86
Acetazolamide(8) 10 mg kg^{-1}	7.37±0.14	6.78±0.12	0.07±0.02	8.13±0.65
Furosemide(8) 0.5 mg kg^{-1}	16.43±0.65	18.94±0.71	0.15±0.05	7.12±0.48
Acetazolamide 10 mg kg^{-1} + Furosemide(8) 0.5 mg kg^{-1}	10.86±0.49**	13.27±0.68**	0.08±0.03**	7.72±0.37

* number of experimental animals ** $p<0.05$
$U_{Na^+}\cdot V$: Urinary sodium ion excreted. $U_{Cl^-}\cdot V$: Urinary chloride ion excreted.
$U\cdot V$: Urine volume. U_{pH}: Urinary pH

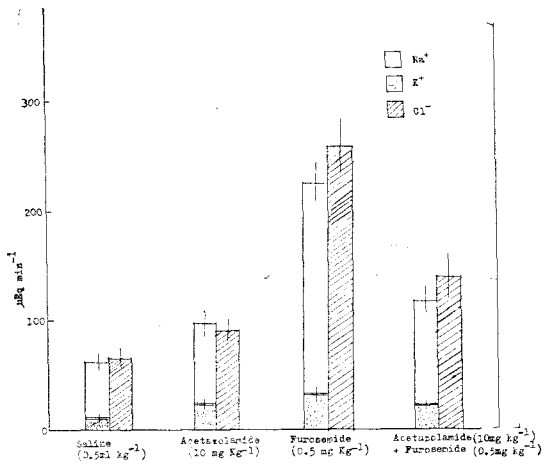

Fig. 2. Urinary electrolytes output over 10 minutes after administration of drugs in the rabbit. (Mean±S.D., n=8)

Table 1 shows the fractional excretion rate of urinary sodium, chloride and urine flow at 10 minutes period after administration of drugs. All parameters show significant reduction after combined administration of acetazolamide and furosemide compared with those of single furosemide group, but fractional excretion rate of urine flow was reduced approximately to the half value of the the furosemide group, and the reduction in the fractional excretion rate of sodium was greater than that of chloride.

Table 2 shows the comparative ratio of the output of sodium and postassium ions versus the output of chloride ion during 10 minutes after injection of drugs. The ratio did not change significantly after the combined administration of two drugs compared with that of single furosemide group.

Table 2. Comparative ratio of the urinary output of sodium and potassium ions versus chloride ion during 10 minutes after administration of drugs

Drugs (I.V.)	$\frac{U_{Na^+ + K^+}}{U_{Cl^-}}$
Saline(8)* 0.5 ml kg^{-1}	0.98±0.02
Acetazolamide(8) 10 mg kg^{-1}	1.14±0.04
Furosemide(8) 0.5 mg kg^{-1}	0.83±0.03
Acetazolamide 10 mg kg^{-1} + Furosemide(8) 0.5 mg kg^{-1}	0.86±0.03

* Number of experimental animals

Discussion

It is generally accepted that there are four principal sites in the nephron where various diuretics exert their actions. Site 1; the proximal nephron which includes the proximal convoluted tubules and pars recta, site 2; the medullary diluting segment comprising the thick ascending limb of Henle, site 3; the cortical diluting segment, and site 4; the collecting duct which contributes to both urinary concentration and dilution. The sites of action of various diuretics may be determined by

comparing the clearance of suitable substances (Eknoyan et al., 1978).

The principal site of action of acetazolamide is at site 1, whereas furosemide is considered to be site 2 diuretic. The primary action of furosemide appears to be inhibition of the active transport of chloride at the luminal border of the tubule of the ascending Henle's limb(Burg, 1976). The action site of acetazolamide was originally attributed to the distal tubule, however current evidence indicates the effect is greater on the proximal tubule than on the distal segment, with little effect on the ascending limb(Goldberg, 1973). Carbonic anhydrase is probably located at the luminal border of the cells of the proximal tubule but not of the distal tubule. Therefore, inhibition of the enzyme may lead to transient changes in pH gradients that limit tubular secretion of hydrogen ion(Rector, 1973). The results show that the increment in the rate of excretion of urine volume, sodium and chloride by furosemide is markedly inhibited by the combined administration of acetazolamide, and the fractional excretion rate of urine flow was reduced almost to the level of single acetazolamide administration. Comparative ratio of the urinary output of sodium and potassium ions versus chloride ion was least affected. Urinary pH after combined administration of furosemide and acetazolamide was higher than that of furosemide group, but lower than that of acetazolamide group.

Chloride concentrations along the nephron depend on acid base equilibrium, most probably according to reciprocal relationship with bicarbonate reabsorption, and whenever bicarbonate reabsorption is absolutely or relatively impaired chloride concentrations in the nephron would decrease(Malinic et al., 1970). On the other hand, Maude(1974) reported that active transport of sodium bicarbonate stimulates sodium chloride reabsorption by generating and maintaining concentration gradients favoring passive reabsorption of this salt. There was a report that the diuretic effect of furosemide was significantly reduced when bicarbonate solution was infused in rabbits(Kim & Cho, 1980).

It is difficult to state the inhibitory action of acetazolamide on the furosemide diuresis is whether direct action or indirect influence by the alteration of the luminal electrolytes composition in the nephron. It can be speculated that the increased concentration of tubular bicarbonate ions induced by acetazolamide interferes the inhibitory action of furosemide on the chloride reabsorption in the ascending Henle's limb. On the other hand, partial blockade of furosemide reuptake from luminal side in the ascending limb due to altered pH induced by acetazolamide may reflect the overwhole reduction of diuretic response of furosemide. However, direct inhibitory effect and other alternative explanations cannot be excluded.

Summary

Effect of acetazolamide on the diuretic action of furosemide was investigated in rabbits. The rates of urine flow and excretion of salts were significantly reduced when furosemide (0.5 mg/kg) was administered with acetazolamide (10 mg/kg) compared to the diuretic response of the single furosemide (0.5 mg/kg) administration. Reduction in the fractional excretion rate of urine volume was more pronounced than the fractional excretion rate of salts. The results suggest that reduction of diuretic action on furosemide by combined administration of acetazolamide is probably due elevated urinary pH and interference in the mechanism of inhibition of chloride transport in the ascending Henle's limb.

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