방사선 방어학회지
제15권 제2호 1990

■論 文■

Inhibition of $Na^{+}-K^{+}$ Adenosine Triphosphatase Activity in Fisher Rats by Uranyl Nitrate

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ABSTRACTS

An attempt was made to test the possibility of a major role for the Na^+-K^+ adenosine triphosphatase (ATPase) system in the diuresis induced by uranyl nitrate(UN). Fisher 344 rats were intravenously injected with UN(5 mg/kg, 15 mg/kg and 30 mg/kg). Urinary excretion of Na^+ and K^+ significantly increased in 24 h exposure on the UN and then decreased below the normal level 3 days after the treatment. Na^+-K^+ ATPase activity of kidney was significantly inhibited in high dosages of UN 15mg/kg and UN 30 mg/kg 3−5 days after injection. And then the recovery of the enzyme activity was observed within 5-10 days after injection, at which the regeneration of the tubular cells occurred.

I. Introduction

The toxic action of uranium on Kidneys is not fully understood, but several potentially important, interrelated events have been identified. Binding of uranium to the brush-border membrane in the distal portion of the proximal tubules may result in reduced reabsorption of Na^+ and, consequently, abnormal electrolyte excretion, proteinuria, glucosuria, aminoaciduria, tubulr necrosis and eventually anuria, even before there is significant structural damage to renal tubular cells [1-4]. Later, disruption of plasma membrane may lead to more extensive changes in membrane transport [5,6].

Na^+-K^+ ATPase are involved in the active transport of Na^+, K^+ and other substances. In the proximal tubule, Na^+-K^+ ATPase activity is strategically located at the peritubular border, the site of the active transport of sodium with a passive reabsorption of water and chloride[7,8]. Uranium inhibits Na^+-K^+ ATPase at the Na^+ site on the enzyme *in vitro*[9]. We previously obtained that Na^+-K^+ ATPase activity was slightly inhibited and recovered in Sprague Dawley rats injected with UN. But Na^+-K^+ ATPase activity and Na^+-K^+ excretion were not closely related[10]. In this report, Fisher 344 inbred rats were used to measure the activity of Na^+-K^+ ATPase. Na^+-K^+ ATPase of Fisher 344 rat was severely inhibited more than that of Sprague Dawley rat.

II. Materials and Methods

Male Fisher 344 rats, weighing 150g-170g, were

This work was supported by the Korean Ministry of Science and Technology Grant.

fed ad libitum a synthetic diet based on NIH-7-open formular and water. Rats were housed in individual metabolic cages. The rats were injected with 5mg, 15mg, 30mg of uranyl nitrate－$UO_2(NO_3)_2 \cdot 6H_2O$, Art 8476, E. Merck－per ml of 5% $NaHCO_3$ per kg body weight, through the tail veins.

Individual rat's body weight and water intake were measured daily. Excretion of Na^+ and K^+ in urine was detected by flame photometer (IL 433, Instrument laboratory).

The microsomal fraction were prepared by the slightly modification of Phillipson [11]. The rats injected with UN were killed, the kidneys were rapidly removed, cooled and weighed. All subsequent steps were performed at 0-4 ℃. The kidneys were minced and homogenized in homogenization buffer (0.25M sucrose, 30 mM imidazole, 5 mM Tris.Cl, pH 6.8, 5mM EDTA, 0.1% (v/v) Triton X-100). The crude homogenates were centrifuged at 8,000g for 20 min. The supernatant was frozen for 24h. The frozen homogenates were thawed and centrifuged at 10,000g for 10 min. The supernatants were centrifuged at 100,000g for 60 min in a Beckman L8-70 ultracentrifuge. The pellets were resuspended in TE buffer (125 mM Tris.Cl pH 7.2, 1 mM EDTA).

Na^+-K^+ ATPase activity was determined by the method of Phillipson [11]. The total ATPase was measured in 100mM NaCl, 20mM KCl, 3mM Na_2 ATP, 5mM $MgCl_2$, 20mM imidazole, 5mM NaN_3, 50mM Tris.Cl, pH 7.4, 0.01mM EDTA, and 50-200 μg microsomal protein. Tandem tubes contained 1mM ouabain. The tubes were incubated for 15 mM at 37℃ and the reaction was terminated by addition of 0.3 ml of 30% (w/v) ice cold trichloroacetic acid. The mixture was centrifuged at 6,000g for 5 min and the Pi content of the supernatant determined. Na^+-K^+ ATPase activity was taken as that fraction of the total ATPase activity that was ouabain inhibitable.

III. Results

Fisher rats were injected with UN (5 mg/kg, 15 mg/kg, 30 mg/kg) and the urines were collected for 5 days. The changes of Na^+ concentration and Na^+ amounts in the urines were measured (Fig. 1) Twenty-four hours after UN injection, the Na^+ concentration in rat urines began to decrease and 4 days after, diluted to 1/6-1/8 as compared to that of the control rats. There were hardly differences between the groups. The Na^+ amount in urine increased 24h after UN injection. Rats treated with UN 30 mg/kg significantly increased Na^+ excretion after UN injection, which returned to the level of control 2-3 days after

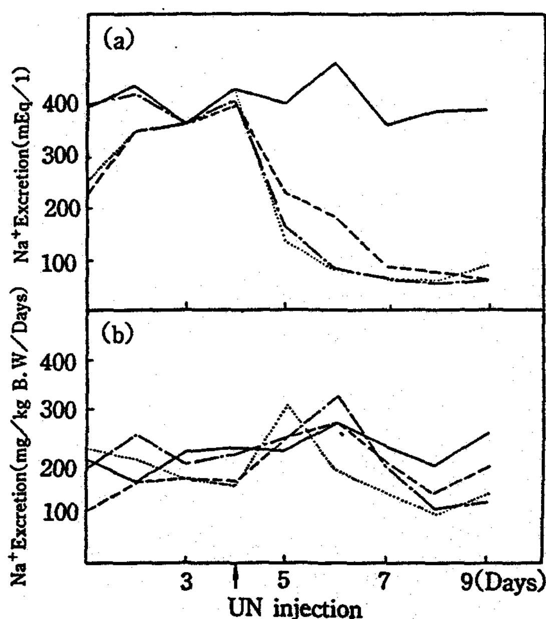

Fig. 1. Effect of uranium poisoning on urinary Na^+ excretion of Fisher rats. Fisher rats were intravenously injected with 5% $NaHCO_3$(——), UN 5 mg/kg (----), UN 15 mg/kg(—・—), UN 30 mg/kg (・・・・・) and measured the concentration(a) and the amount(b) of Na^+ excreted with urine.

UN injection and then decreased below the control value. In rats treated with UN 5 mg/kg and UN 15 mg/kg, the excretion of Na^+ increased after 24 h, reached maximum 48 h after UN injection and then decreased below the control.

The changes of K^+ concentration and K^+ contents in the urines were measured in Fisher rats injected with UN (Fig.2). The changes of K^+ concentration after UN injection were similar to those of Na^+ concentration. Rats treated with UN excreted dilute K^+ in urine. The K^+ concentration in urine decreased to 1/4-1/6 of control value. There were not big differences among the groups

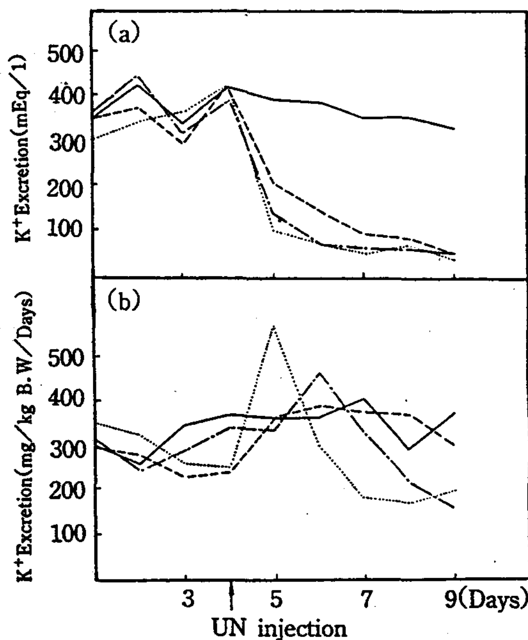

Fig. 2. Effect of uranium poisoning on urinary K^+ excretion of Fisher rats. Fisher rats were injected with UN as the Legend of Fig. 1. The concentration(a) and the amount (b) of K^+ excreted with urine were miasured.

but high dosage of UN leads to more dilute K^+ excretion than low dosage one. The amount of K^+ excreted in urine was measured. In rats treated with high dosage of UN maximum excretion of K^+ were shown 24h after UN injection and then decreased. Three days after UN injection , the amount of K^+ excretion was less than that of control. Rats treated with UN 5mg/kg and 15 mg/kg increased the K^+ excretion after UN injection, maintained for 2-3 days and decreased below the control.

We investigated the relation among the increase of Na^+, K^+ excretion and urine volume (Fig.3). The excretion of Na^+, and K^+ were changed in proportion to the urine volume in control rats. Also in the rats treated with UN 5 mg/kg, Na^+ and K^+ excretion increased in proportion to the increase of urine volume but 3-4 days after UN injection decreased inversely proportional to the increase of urine volume. However, in the rats treated with UN 15 mg/kg and UN 30 mg/kg, the excretion of Na^+ and K^+ increased in proportion to urine volume and decreased in proportion to that. From these results, the changes of the Na^+ and K^+ excretion were not the same as those of urine volume but closely related to the urine volume.

The Na^+-K^+ ATPase system plays a major role in transepithelial Na^+ transport. It has been known for some time that cardiac glycosides are inhibitors of Na^+-K^+ ATPase [12]. The natriuresis induced by large doses of cardiac glycosides is accompanied by a reduction of Na^+-K^+ ATPase activity in the kidney[12]. The activities of Na^+-K^+ ATPase were measured in microsomal fraction of entire kidneys 1-10 days after UN injection into the teil veins of Fisher rats (Table 1). Three days after the treatment, the enzyme activities were markedly less in kidneys at the higher dosages of UN 15 mg/kg and UN 30 mg/kg than those of kidneys from rats not treated. But the enzyme activities were not changed by low dosages

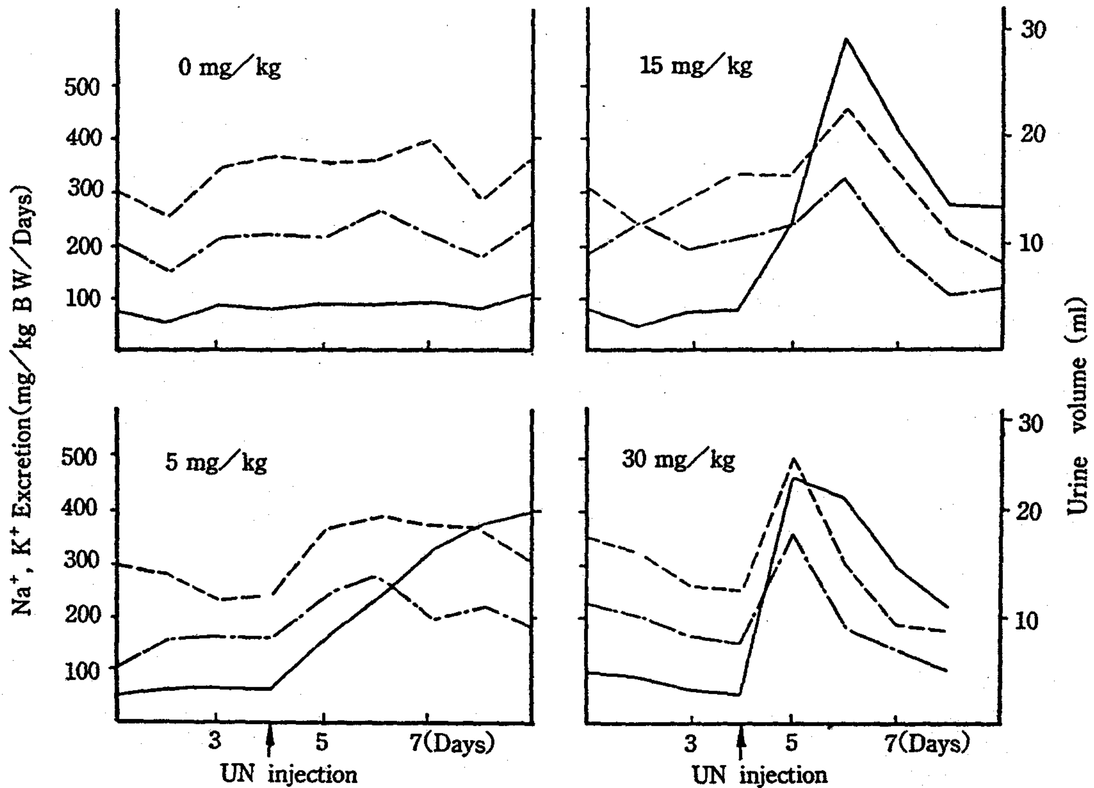

Fig. 3. Correlations among the urine, Na^+ and K^+ excretion in Fisher rats after UN injection. Fisher rats were injected with UN as the legend of Fig. 1. and compared the relation with urine volume(———), Na^+ (—・—) and K^+ excretion(-----).

of UN 5 mg/kg. The recovery of the enzyme activity was also observed, 5 days after the treatment of UN 15 mg/kg and 10 days after the treatment of UN 30 mg/kg.

Discussion

Uranyl nitrate causes acute tubular necrosis, diuresis and repeated exposure to uranium com-

Table 1. Changes of Na^+-K^+ ATPase in the kidney of Fisher rats*

Treatment of U.N. (mg/kg BW)	Na^+-K^+ ATPase(μmol Pi $mg^{-1}hr^{-1}$) *			
	1 day	3 days	5 days	10 days
30	42.8	12.8	9.5	29.3
15	39.5	15.5	36.9	ND
5	38.8	35.9	29.8	30.3
0	37.4± 5.4	37.4± 5.4	37.4± 5.4	37.4± 5.4

** Fisher rats were injected with UN as the legend of Fig. 1 and the kidneys were rapidly removed, 1 day, 3 days, 5 days, 10 days after UN injection.*

Na^+-K^+ ATPase were measured in microsomal protein, 50—200μg, of kidney homogenates.

pounds can produce renal tubular degeneration, necrosis, atrophy, interstitial fibrosis, and glomerular damage [13,14]. The kidney contains a very high activity of Na^+-K^+ ATPase considered as a target of diuretics. We, therefore, evaluated whether the diuresis and natriuresis in Fisher 344 rats induced by UN related to the changes of Na^+-K^+ ATPase in the kidney.

Fisher 344 rats were intravenously injected with UN (5 mg/kg, 15 mg/kg, 30 mg/kg). Urinary excretion of Na^+, K^+ and urine volume were chected daily. Within 24h after injection, the excretion of Na^+, K^+ and the volume of urine increased(Fig. 1,2) but Na^+-K^+ ATPase was not changed (Table 1). Three five days after UN injection, the excretion of Na^+ and K^+ decreased under the control as urine volume decreased. However, in the rats treated with UN 30 mg/kg, 15 mg/kg, the Na^+-K^+ ATPase was 30% of control activity 3 days after injection and the enzymeactivitiesin UN 15 mg/kg recovered 5 days after injection, in UN 30 mg/kg, 10 days after injection. In UN 5 mg/kg, the activities of Na^+-K^+ ATPase were not changed, but the excretionsof Na^+ and K^+ were varied on the intervals of UN treatment. As discussed above, the variation of Na^+ and K^+ excretion did not have any significant relation with the activities of Na^+-K^+ ATPase. These findings do not agree with Nechay's proposal[8]for the biochemical basis of diuretic actions in rat that uranium inhibits Na^+-K^+ ATPase at the Na^+ site on the enzyme, in vitro. Differences between two systems(*in vivo and in vitro*)may affect the results.

We previously reported that the destruction of mitochondria was observed in the proximal tubular cells 24h after UN injection [15]. Therefore mitochondrial oxidative phosphorylation is inhibited by the injection of UN (5 mg/kg or more) and mitochondria in the proximal tubular cells can not supply ATP for the reabsorption of Na^+ and water 24h after UN injection[15]. Although the Na^+-K^+ ATPase is intact 24h after UN injection (Table 1), the enzyme activity may be inhibited by ATP deficiency or uranium present in the tubular cells. So the Na^+ reabsorption decreases. The initial stage of acute tubular necrosis is partial degeneration of the proximal tubular epithelium within 24h, followed by frank necrosis until 72h, then active regeneration of the tubular cells is seen after 5-10 days, and finally dilated tubules lined with epithelial cells are regenerated[15]. Our findings suggests that the inhibition of Na^+-K^+ ATPase 3-5 days after UN injection may due to the necrosis of tubular epithelial cells which are the major site of Na^+-K^+ ATPase. Also the recovery of the enzyme seems to be due to the regeneration of the tublur epithelium.

It was observed that Na^+-K^+ ATPase in Sprague Dawley rats was slightly inhibited only in high dosage of UN 30 mg/kg[10].But the enzyme activity in Fisher 344 rats was remarkably inhibited. Excretions of Na^+, K^+ and urine outputs in Fisher rats also varied more rapidly and signficantly than those of Sprague Dawely rats. So, Fisher rats were easily poisoned by UN and seemed to be useful to observe the biochemcal changes induced by UN.

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