Low-Molecular-Weight Proteins in Urine from Rabbits Given Nephrotoxic Compounds

Kazuo NOMIYAMA, Hiroko NOMIYAMA and Mamoru YOTORIYAMA

Department of Environmental Health, Jichi Medical School
Minamikawachi-Machi, Tochigi, 329-04 Japan

(Received July 10, 1981 and in revised form October 23, 1981)

Abstract: The nature of low-molecular-weight proteinuria, which were observed in rabbits given nephrotoxic compounds such as cadmium, kanamycin, uranium, mercury or chromium, were studied in the present experiment: rabbits were given subcutaneous injections of cadmium 6 times a week at a dose level of 0.5 mgCd/kg over a period of 32 weeks. Main components of proteins in urine of cadmium-treated animals were proteins of molecular weight 67,000 (probably albumin) and higher-molecular-weight, and that might suggest the existence of tubular dysfunctions as well as of glomerular dysfunctions. Rabbits given a single injection of uranium, chromium or mercury indicated low-molecular-weight proteinuria as well. Molecular weight distribution of proteins in urine of the uranium-, chromium- or mercury-treated rabbits were hardly distinguishable from that of cadmium-treated rabbits. Low-molecular-weight proteinuria appeared in spite that tubular reabsorption of phosphorus was higher than 80%. The mechanism of low-molecular-weight proteinuria was discussed.

Keywords: Cadmium—Uranium—Chromium—Mercury—Rabbit—Low-molecular-weight proteinuria—Tubular dysfunction

INTRODUCTION

β₂-microglobulin (MG) of molecular weight (MW) 11,800 and retinol-binding protein (RBP) of MW 21,400 have been determined in concentrations of above 10 and 100 mg/l in urine from patients with renal tubular dysfunctions\(^1,2\) as well as above 50 and 40 mg/day from urine of patients with cadmium poisoning\(^2,3\). The screening for cadmium-induced tubular dysfunctions, therefore, have been performed by determining above low-molecular-weight (LMW) proteins in urine\(^4\). However, very slight urine MG increase, which were determined by the radio-immunoassay technique as low as 3 μg/l, was sometimes misunderstood as tubular dysfunction, in spite of the fact that urine MG increases to above several thousands μg/l in cases of tubular dysfunctions\(^1\). Urine MG was elevated temporarily in
exercises as well\(^6\). Further, the ageing enhanced urine MG\(^7\)\(^-\)\(^9\), sometimes without any renal dysfunctions\(^9\)\(^,\)\(^10\). Therefore, the elevation in urine MG in residents in cadmium-polluted areas cannot be attributed only to cadmium-induced renal dysfunctions\(^11\).

The present authors intended to study on the nature of LMW protein in urine from rabbits given nephrotoxic compounds such as maleic acid, kanamycin, uranyl acetate, potassium dichromate, mercuric chloride and cadmium chloride for establishing the differential diagnosis of chemical-induced renal dysfunctions.

Materials and Methods

Eighteen 4-month-old male rabbits of Japanese white strain weighing 2.7 kg were maintained in environmental temperature of 22°C, humidity of 55% and daylight between 7 a.m. and 7 p.m. by feeding commercial pelleted food (CLEA CR-3) and water ad libitum. Four rabbits were given subcutaneous injections of cadmium chloride 6 times a week at a dose level of 0.5 mgCd/kg over a period of 32 weeks, 2 rabbits a single subcutaneous injection of maleic acid at a dose level of 400 mg/kg, 4 rabbits 3 intramuscular injections of kanamycin every other day at a dose level of 1.0 g/kg, 3 rabbits a single venous injection of uranyl acetate at a dose level of 0.2 mgU/kg, 3 rabbits a single subcutaneous injection of potassium dichromate at a dose level of 1.77 mgCr/kg and 2 rabbits a single subcutaneous injection of mercuric chloride at a dose level of 1.0 mgHg/kg, respectively. Body weight, blood and 24-hours urine were examined at intervals: urine protein and amino acids were determined by Tsuchiya-Biuret method\(^12\) and trinitrobenzene sulfonate method\(^13\). Urine glucose was tested with Tes tape (Shionogi Pharmaceut. Co.). Creatinine clearance (C\(_{Cr}\)) and tubular reabsorption of phosphorus (% TRP) were computed from urine and plasma creatinine and inorganic phosphorus determined by Jaffe method\(^14\) and Fiske-Subbarow method\(^15\). LMW proteins in urine were analyzed by sodium dodecyl sulfate (SDS) polyacrylamide-gel electrophoresis\(^16\).

Results

1. General Health Conditions

Two rabbits of the maleic acid group died within 24 hours. Pyonephrosis was found in all animals given maleic acid. Two out of 4 of the kanamycin group died within 4 days after kanamycin administration. Extensive hemorrhage was observed in the kidneys.

Body weight of rabbits given cadmium increased slightly until the 8th–14th week, and then decreased afterwards.
2. **Urine Protein**

Protein of the cadmium group increased after 12 weeks and elevated remarkably after 15 weeks on an average. Protein of the kanamycin group was elevated.

![Fig. 1. Urinary excretion of proteins in rabbits given cadmium, maleic acid, kanamycin, uranium, chromium or mercury. Every mark represents each experimental animal.](image-url)
instantly to 1,000 mg/day after kanamycin administration, but decreased soon to the normal level. Protein of the uranium group increased slightly after uranium administration to 100–200 mg/day in the 4th day, and then decreased afterwards. The chromium group indicated an elevation in protein up to 250 mg/day in the 2nd day and then a decrease later. One rabbit of the mercury group showed also an elevation to as high as 600 mg/day in the next day, but recovered to the normal level soon.

3. **Urine Glucose**

Urine glucose was detected in the 12th–15th week in the cadmium group. It was also detected in the uranium and chromium groups. On the other hand, urine glucose was not detected in rabbits given maleic acid, kanamycin or mercury.

Fig. 2. Urine glucose (qualitative).
4. Urine Amino Acids

Urine amino acids increased in the 9th–12th week in some rabbits of the cadmium group. Amino acids of the kanamycin group were elevated remarkably to 15–25 m moles/day in the 2nd day. Amino acids of the uranium, chromium and mercury groups indicated temporal increases in the 6th–7th day.

Fig. 3. Urinary excretion of aminoacids.
5. Creatinine Clearance ($C_{cr}$)

Creatinine clearance was unchanged in the cadmium group. Some rabbits of the kanamycin group showed a decreased creatinine clearance in the 4th day. Creatinine clearance of the uranium group also indicated the remarkable decrease in the 4th day, and did not recover to the normal level within 12 days. The mercury group indicated also a temporal decrease in the 2nd day. The chromium group did not show any changes in creatinine clearance.

6. Tubular Reabsorption of Phosphorus (% TRP)

The cadmium group did not indicate any changes. Kanamycin and mercury did not change tubular reabsorption of phosphorus as well. Some rabbits of the
Fig. 5. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoretic patterns of urine proteins of one rabbit each from cadmium, maleic acid, kanamycin, uranium, chromium and mercury groups.
uranium group showed a remarkable but temporal decrease to 87% in the 4th day, and then recovered to the normal level by the 12th day. One out of 3 rabbits of the chromium group indicated a slight decrease (97.4%) in the 4th day, but recovered to the normal level by the 12th day.

7. Molecular Weight Distribution of Proteins in Urine

In the cadmium group, proteins of MW 12,000 and 67,000 were found during the 6th–9th week. Slight amount of protein of MW 25,000 was detected during the 16th–19th week. The ratio of protein of MW 67,000 increased with the increase in cadmium administration, and higher-molecular-weight (HMW) protein was also detected after the 12th week.

In the maleic acid group, protein of MW 67,000 was detected in the 2nd day. In one rabbit of the kanamycin group, LMW proteins (MW 12,000 and 25,000) were found in the 1st day, but disappeared soon. The main components of urine protein were those of MW 67,000 and higher molecular weight. In the uranium group, LMW proteins of MW 12,000 and 25,000 were found 4 days after uranium administration, but disappeared soon. The main components of urine proteins were those of MW 67,000. In urine of the chromium group, massive proteins of MW 67,000 were found as well as LMW proteins of MW 12,000 and 25,000 in the 1st–3rd day. After the 6th day, most urine proteins were MW 67,000 and higher molecular weight, and scarce amount of LMW proteins of MW 12,000 were detected in some rabbits. In the mercury group, massive amount of LMW protein of MW 12,000 was also found in urine in the 1st day. Lesser amount of protein of MW 67,000 was determined as well. In the 3rd day, LMW proteins of MW 12,000 was still detectable in urine from 1 out of 2 rabbits. LMW protein was undetectable after the 6th day.

DISCUSSION

1. Low-Molecular-Weight Proteinuria caused by Nephrotoxic Compounds

LMW proteinuria of the cadmium group was hardly distinguishable from those of the uranium and chromium groups, in spite of some slight differences in the degree among them. Because uranium and chromium were given to animals only once, LMW proteinuria disappeared soon in the recovery from renal dysfunctions. The above data might indicate that the nature of urine proteins in heavy metal intoxications is basically identical to each other.

Because electrophoretic mobility of urine proteins from rabbits given cadmium was distinctly different from those of rabbits given uranium\(^\text{17}\), Friberg et al.\(^\text{18}\) claimed that renal dysfunction in rabbits given cadmium was different from that of rabbits given uranium, and that they could not accept Nomiyama's paper\(^\text{19}\) on the mechanism of urinary excretion of cadmium, because of Nomiyama's using uranyl-treated animals as a renal dysfunction model. Our findings in the present
study disagreed to the original finding of Friberg\textsuperscript{17}, probably because Friberg did not compare molecular weight of these LMW proteins. The present authors can not accept the criticism of Friberg \textit{et al.}\textsuperscript{18} at this stage.

In the present experiment, extensive proteinuria, especially LMW proteinuria, was observed in rabbits given nephrotoxic compounds, in spite of little depressions in creatinine clearance and tubular reabsorption of phosphorus. Consequently, LMW proteinuria might be one of good indices for screening the early health effect of heavy metals prior to the appearance of renal dysfunctions.

\(\beta_2\)-microglobulinuria (MGuria) was observed in Minamata disease patients\textsuperscript{20} as well as in mercury workers and mercury poisoning patients\textsuperscript{21,22} in spite of disagreement with the observation by Roels \textit{et al.}\textsuperscript{23} on 17 workers exposed to mercury for 5 years on an average. Mercury was also reported potently to stimulate human lymphocytes to produce MG \textit{in vitro}\textsuperscript{24}. In addition, temporal elevations in plasma and urine MG were observed in new cadmium workers after 3 months exposure to cadmium, when any renal dysfunctions were detected\textsuperscript{25}. Therefore, the mechanism of MGuria in cadmium health effects is unable to be elucidated only by the renal tubular dysfunctions as mentioned earlier by the present authors\textsuperscript{11}. It is still necessary for us to clarify the mechanism of proteinuria due to various kinds of chemicals.

2. \textit{Low-Molecular-Weight Proteinuria as An Early Sign of Cadmium Health Effects}

As shown in the present experiment, LMW proteinuria and very slight proteinuria were found in the 6–9th week of cadmium administrations. Aminoaciduria was detected in the 9–12th week, and proteinuria and glycosuria in the 12–15th week. The above results quite accorded with our previous results\textsuperscript{12}. LMW proteinuria was followed by aminoaciduria and then by proteinuria and glycosuria in cadmium intoxication. Therefore, LMW proteinuria and aminoaciduria might be good index for detecting renal tubular dysfunctions in an early stage of cadmium intoxication.

3. \textit{Cadmium-induced Renal Dysfunction—Only Tubular Dysfunction?}

Main components of urine proteins were albumin and HMW proteins. This fact might suggest that cadmium affects not only renal tubuli but also renal glomeruli. The probable existence of renal glomerular dysfunctions was also suggested in our report\textsuperscript{25}, in spite that the glomerular filtration rate (creatinine clearance) was not always depressed.

\textbf{Acknowledgement}

This study was supported by Japan Ministry of Health and Welfare's Contract Fund of Food Sanitation Research in 1979, and Gunma-Ken Department of Health
K. NOMIYAMA, H. NOMIYAMA AND M. YOTORIYAMA

and Environment.

REFERENCES