Alterations of Metal Content in the Kidneys of Puromycin Aminonucleoside-Administered Rats

Makoto FUKATA, Taiji HAYASHI, Jun TERUI, and Jun-ichi SUDO*

Department of Clinical Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-02, Japan. Received January 6, 1994; accepted March 17, 1994

To explore the mechanism responsible for puromycin aminonucleoside induced-nephrotoxicity, metal content was preliminarily investigated in the kidneys of rats that had received a single subcutaneous injection of the agent (80 mg/kg body weight). In the group that received this agent, the renal content of Fe rose on days 5, 10, and 15; that of Cu fell on days 10 and 15; that of Zn rose on day 5; that of Se fell on day 10; there were no changes in Mn throughout the experimental period. Providing that these findings directly involved the renal activities of superoxide dismutases and glutathione peroxidase, the renal reduction of Cu and Se gave proof counter to previous findings of an enzymatic protective system against possible attacks by the oxygen radicals.

Keywords puromycin aminonucleoside; kidney; toxicity; copper; selenium; rat

Puromycin aminonucleoside produces glomerular proteinuria, the major component being albumin.1-3) The main target of this agent is on the glomeruli.1-3) Lipid peroxidation ascribable to oxygen radicals produced in the kidney has recently been proposed to be one of the mechanisms responsible for this puromycin aminonucleoside-induced renal injury.4-7)

With regard to lipid peroxidation, iron (Fe) plays a role in the production of hydroxyl free radicals and singlet oxygen from superoxide anions and hydrogen peroxide, through the Fenton reaction.9) Enzymes that play roles in producing and scavenging oxygen radicals are known to possess metals in their chemical structures: molybdenum (Mo) in xanthine oxidase10); copper (Cu), manganese (Mn) and zinc (Zn) in superoxide dismutase11); and selenium (Se) in glutathione peroxidase.12,13)

Based on these findings, we hypothesized that if puromycin aminonucleoside-induced renal injury was attributable to lipid peroxidation, the content of the abovementioned metals would be altered in kidneys in animals subjected to treatment with the agent. Herein, we preliminarily carried out experiments in an attempt to confirm this hypothesis.

MATERIALS AND METHODS

General Procedures Male Wistar rats (Sankyo Labo Service; Tokyo), weighing 200–220 g, were used. Rats received a single subcutaneous injection of puromycin aminonucleoside (Sigma Chemicals; St. Louis, U.S.A.) at 80 mg/kg body weight, on the first day of the experiment.14) Control rats received a single injection of an equivalent volume of saline given in the same manner. The day of the administration of the agent was designated day 0.

Kidney samples were obtained at 0 (before), 5, 10 and 15d following the administration. The animals were anesthetized with ether, and the abdominal cavity was opened through a ventral incision. Both kidneys were then flushed with saline via the abdominal aorta to wash away the residual blood, after which they were removed. The left kidney was weighed and homogenized with 0.25M sucrose in a glass/Teflon Potter homogenizer.

Analytical Procedures The total protein concentration in the renal homogenates was determined spectrophotometrically using the modified method15) of Lowry et al.16) The renal homogenates were wet-digested with perchloric and nitric acids. The concentrations of metals (Cu, Fe, Mn, Se and Zn) in the mineralized homogenates were determined with a Hitachi Z-8100 polarized Zeeman atomic absorption spectrophotometer with a graphite furnace apparatus (Hitachi, Tokyo); of note, Se was determined by raising its sensitivity by the addition of nickel.17)

Statistics Results were expressed as means ± S.E.M. The data were subjected to analysis of variance, and subsequently to the unpaired Student's t-test, with p values of less than 0.05 being considered significant.

RESULTS

As compared with the control group, the puromycin aminonucleoside-administered group showed increases in Fe, as a plateau, from days 5 to 15 (Fig. 1). A reduction in Se was observed in the administered group on day 10 (Fig. 1). The administered group showed linear reductions in Cu from days 10 to 15 (Fig. 2), and, in contrast, an increase in Zn on day 5 (Fig. 2). Regarding Mn, there were no significant differences between the two groups throughout the experimental period (Fig. 2).

DISCUSSION

Our previous report18) denoted that albuminuria reached a high and quite steady-state level during the period of days 5 to 15 following the administration of the agent. Based on this finding, experiments in which kidney samples were obtained were carried out on days 0 (before), 5, 10 and 15.

In the present study, metals that could be detected in the kidneys were Fe, Cu, Zn, Mn, and Se, but not Mo. Among those metals, renal Fe content was elevated by puromycin aminonucleoside-administration (Fig. 1). It has been reported that the puromycin aminonucleoside-administration resulted in ferritin deposition in the
glomeruli\textsuperscript{19} and Fe deposition in the collecting tubules.\textsuperscript{20} If the elevation we found in renal Fe content was mainly due to this deposition mechanism, we speculated that the Fe was not necessarily involved in the Fenton reaction.\textsuperscript{8} Nevertheless, since this reaction has been known to occur at extremely low concentrations (in a trace level) of Fe\textsuperscript{3+},\textsuperscript{21} we could not rule out the possibility that the elevated level of renal Fe had advanced the above reaction in the kidney. Accordingly, further investigation is required to determine the extent to which the elevated level of renal Fe is attended by altered levels of free Fe\textsuperscript{2+}, Fe\textsuperscript{3+}, or Fe bound to ferritin and other proteins.

Our study denoted that puromycin-aminonucleoside administration brought the reduction of Cu level and the elevation of Zn level in the kidneys (Fig. 2); the renal level of Mn remained unchanged (Fig. 2). In relation to the three metals, the rat kidneys have been reported to contain two types of superoxide dismutase, in which the metallic composition differs\textsuperscript{11}: Cu,Zn-superoxide dismutase and Mn-superoxide dismutase. Both Cu and Zn have been shown to be critical for the exertion of the activity of Cu,Zn-superoxide dismutase.\textsuperscript{11} Accordingly, providing that these findings directly involved the renal superoxide dismutases, we considered it to be possible that the reduction of Cu in the kidney brought about a reduction in the renal activity of Cu,Zn-superoxide dismutase, and that Mn-superoxide dismutase was hardly influenced in the kidney.

Regarding the depression of the renal level of Se in the puromycin aminonucleoside-administered group (Fig. 1), this Se has been known to be a representative metal contained in glutathione peroxidase, as a selenocysteine residue.\textsuperscript{12,13} Therefore, we considered it possible that the depression of renal Se content influenced the renal activity of glutathione peroxidase; probably, by causing a reduction in the renal activity of the enzyme.\textsuperscript{22}

In conclusion, our findings on all the metal contents apparently disproved the efficacy of an enzymatic protective system against possible attacks by oxygen radicals.

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**REFERENCES**