Nephrotoxicity Induced by Adenine and Its Analogs: Relationship between Structure and Renal Injury

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Twenty-four adenine analogs were administered to mice and the relationship between the structure of analogs and the occurrence of renal injury was examined.

Plasma urea nitrogen (UN) and creatinine levels were measured 24 h after oral administration of analogs. Both levels increased in the adenine-, 8-azaadenine-, isoauanine-, or 6-dimethyl aminopurine (6-DMAP)-administered group, but did not increase in the other analog groups. From light microscopy, the damages of tubuli, mainly of proximal tubuli, were observed in the kidneys of these four groups. The common property of these compounds is the strong basicity of nitrogen which binds the 6-position of the purine ring. Furthermore, UN and creatinine increased time-dependently with intravenous administration of isoauanine. When adenine was intravenously administered, UN slightly increased at 1 h, but creatinine was unchanged. No changes were observed in the 6-DMAP- or 8-azaadenine-administered group.

The basicity of nitrogen which binds to the 6-position of the purine ring is thus considered to be related to the occurrence of renal injury with oral administration, and isoauanine has high affinity with the kidney.

Keywords: adenine; analog; isoauanine; urea nitrogen; creatinine; mouse kidney

Adenine is one of the most important substances in the body, because it is converted into adenosine by the purine salvage enzyme, adenine phosphoribosyl transferase (APRT), and adenine nucleotides are used in the body. Adenine is not only endogenously produced from 5'-methylthioadenosine by methylthioadenosine phosphorlyase, but also exogenously supplied from foods, mainly meats. For example, it is known that adenine rich diets cause a rise in serum uric acid level. These findings suggest that adenine is always taken into the body and used by the body. However, genetic defects in APRT enzyme have been reported, and 2,8-dihydroxyadenine (2,8-DHA), a very insoluble substance, is directly produced from adenine by xanthine oxidase. Furthermore, 2,8-DHA crystalluria and urolithiasis are induced by a deficiency of APRT. On the other hand, an animal model of chronic renal failure was made following lengthy administration of adenine-containing foods. In animals, the renal toxicity of adenine is considered to be caused by the accumulation of 2,8-DHA in the tubuli and by the formation of guanindio compounds. Therefore, xanthine oxidase, which produces active oxygen, has been reported to play a large role in adenine-induced renal injury. We observed that a single oral administration of adenine induced renal injury, and that such occurrence was inhibited by simultaneous administration of 4-aminopyrazolo[3,4-d]pyrimidine (4APP), an adenine analog. The renal injury was not reduced when allopurinol, which is a xanthine oxidase inhibitor, was administered together with adenine to mice, although the production of 2,8-DHA decreased. From these results, the occurrence of renal injury may not be due to the accumulation of 2,8-DHA. Pancreatic injury occurred when adenine was administered together with dimethyl sulfoxide and 4APP also induced pancreatic injury. Therefore, it is thought that adenine induces several types of damage in the body, although it is also one of the most important substances. In the present investigation, we attempted to clarify that relationship between the structure of adenine and renal injury using adenine analogs.

MATERIALS AND METHODS

Animals Six-week-old male ddY strain mice were purchased from Japan SLC Co. (Shizuoka, Japan) and housed for one week prior to the start of the experiments, during which time they were fed standard mouse food (MF, Oriental Yeast Co., Ltd., Tokyo) and given tap water ad libitum.

Chemicals Adenine, 6-dimethylaminopurine (6-DMAP), 8-azaadenine, purine, 2-aminopurine (2-AP), 4APP, oxypurinol, 3-methyladenine, 2,8-DHA, adenine-N1-oxide, 8-azaauanine, and 8-azaxanthine were purchased from Sigma Chemical Co., (MO, U.S.A.). Isoauanine, 6-aminoindazole (6-AI), 2-amino-6-methoxypurine (2-A-6-MP), 2,6-diaminopurine (2,6-DAP), and 8-azahypoxanthine were obtained from Tokyo Chemical Industry Co., Ltd., (Tokyo). Allopurinol, adenosine, inosine, xanthine, hypoxanthine, guanine, and uric acid were purchased from Wako Pure Chemical Industries, Ltd., (Osaka). Figure 1 shows the structures of adenine and its analogs.

Administration of Adenine and Its Analogs The dosage of adenine and its analogs was based on the LD50 value of adenine (745 mg/kg) and our previous report. After one night of fasting, the mice were divided into twenty-five groups of six mice each, and two mice from each group were used in each experiment. They were orally administered a solution (10 ml/kg) containing saline

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(control group), and 175 mg/kg of each drug. Twenty-four hours after each drug was administered, all mice were anesthetized and heparinized blood specimens were collected.

**Intravenous Administration of Adenine, Isoguanine, 6-DMAP and 8-Azaadenine** Lindblad et al.\(^{14}\) reported that the highest intravenous dose of adenine administered to dogs which could be considered safe was 10 mg/kg. We thus intravenously administered 7 mg/kg of adenine, isoguanine, 6-DMAP, or 8-azaadenine to the mice. Thirty minutes, 1, 2, 4, and 8 h after each drug was administered, heparinized blood specimens were collected. In the control group, 0.2 ml of saline was intravenously administered and 8 h later, heparinized blood specimens were collected.

**Observation and Analysis** Plasma urea nitrogen (UN) and creatinine were measured with an automatic analyzer (705, Hitachi Co., Tokyo) by clinical kits using the urease and indophenol method (Nescauto UN; Nippon Shoji Co., Osaka) and the picric acid method (Nescauto CRE; Nippon Shoji Co.).

The method for the measurement of 2,8-DHA\(^{19}\) was modified and renal isoguanine concentration was measured as follows: a 10% homogenate was prepared with 1N NaOH solution from the kidney, and 100 μl of conc. HCl solution was added to 1200 μl of the homogenate. After centrifugation, the supernatant was filtered into a filter unit (0.22 μm; Millex-GV, Millipore Products Div., MA, U.S.A.), and the content of isoguanine was assayed by high performance liquid chromatography (Shimadzu Co., Kyoto, Japan). After filtration, 10 μl was
injected and eluted with a mixture of 50 mM NaH₂PO₄ and acetonitrile (95:5) at a flow rate of 1.0 ml/min on a Cosmosil 5C18-AR column (250 × 4.6 mm i.d., Nakalai Tesque Inc., Kyoto), and the eluent was monitored at 305 nm. Isoguanine and 2,8-DHA were isolated (isoguanine, 3.213 min; 2,8-DHA, 3.400 min) as shown in Fig. 2.

For light microscopy, the kidneys from individual mice were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin (HE).

**Statistical Analysis** All results are given as the mean value ± S.E. Differences among the group were determined by a one-way analysis of variance, and the comparison of means among the groups was evaluated using the method of Dunnett.

**RESULTS**

As shown in Fig. 3, the plasma UN and creatinine levels were higher in the groups administered adenine, isoguanine, 6-DMAP, or 8-azaadenine than in the control group. Both levels in the groups administered the other analogs did not differ from those levels in the control group.

HE preparations showed epithelial flattening, and un-
cleanliness of cytoplasmic fine texture of tubuli, mainly of proximal tubuli, in the kidneys was observed in the isoguanine-administered group. The same findings were made in the mice kidneys in the groups administered adenine, 6-DMAP or 8-azaadenine (Fig. 4).

Time dependent changes in the levels of plasma UN and creatinine were observed when adenine, isoguanine, 6-DMAP, or 8-azaadenine was intravenously administered (Fig. 5). Thirty minutes after isoguanine administration, the creatinine level was significantly higher than that in the control group (0.24 ± 0.03 mg/dl), and this high level was maintained even 8h later. The UN level of the isoguanine-administered group also increased 1h after the administration and showed a time-dependent increase (control group: 19.1 ± 0.9 mg/dl). In the adenine-administered group, the UN level peaked at 1h after the administration, however, in the 6-DMAP- or 8-azaadenine-administered groups, the UN and creatinine levels did not differ from those in the control group.

There was possibly some isoguanine in the kidney with the oral administration of adenine, because the retention time of this analog coincided with that of standard isoguanine. Ten hours after the administration, isoguanine was detected 40.1 ± 10.7 μmol/g tissue, but 2,8-DHA was not found. However, 24h after the administration, the retention time of the peak was detected close to the peak of the standard 2,8-DHA. In the kidneys of control group, there were no peaks of isoguanine or 2,8-DHA.

DISCUSSION

As shown in Fig. 1, twenty-four adenine analogs which
were used for this experiment have purine, pyrazolopyrimidine, or triazolopyrimidine rings and possess amino, carbonyl, hydroxy, methyl, or methoxyl groups. From the results shown in Figs. 3 and 4, the compounds with purine or triazolopyrimidine rings induced renal injury, especially tubular damage. A nitrogen is apparently required which binds to the 6-position of the purine or triazolopyrimidine rings. On the other hand, 4APP, which has a pyrazolopyrimidine ring, did not induce renal injury. The basicity of the nitrogen which binds to the 6-position of the purine ring increases because adenine and 8-azaadenine possess nitrogen which is positioned ortho of the amino group, but the basicity of the amino group of 4APP does not increase because the amino group is at the meta position. Therefore, the basicity of the nitrogen at the 6-position in the four compounds which induced renal injury is higher than that of the other analogs, and the basicity of the nitrogen at the 6-position may be necessary to induce renal injury.

Whether or not these four compounds directly induce renal injury was examined by their intravenous administration to mice. The UN and creatinine levels in the iso- guanine-administered group increased time-dependently, as shown in Fig. 5. However, UN increased only slightly in the adenine-administered group, and neither level changed in the 6-DMAP- or 8-azaadenine-administered group. As 10 mg/kg of adenine did not differ pathologically from the control in dog, it is thought that 7 mg/kg of adenine was the highest safe dose in mouse. Three possibilities were therefore considered: (1) Iso guanine has high affinity with the kidney. (2) Iso guanine directly induces renal injury. (3) One of the metabolites of adenine, 8-azaadenine or 6-DMAP induces renal injury. When xanthine oxidase directly acts on adenine, adenine is converted into 8-hydroxyadenine and then into 2,8-DHA. Adenine has also been reported to be converted into 6-N-hydroxylaminopurine in hepatic microsomes, and it may be converted into 2-hydroxyadenine, 2,6-DAP, which has an amino group instead of a hydroxy group of 2-hydroxyadenine, did not show an increased UN or creatinine level (Fig. 3), and the amino and hydroxy groups at the 2-position did not have electron efficiency. Moreover, although 2,8-DHA did not induce renal injury, the basicity of the nitrogen at the 6-position was reduced by two hydroxy groups (Fig. 3). However, the ketone group at the meta position from the amino group increased the basicity of the nitrogen. Iso guanine and 2-hydroxyadenine are keto-enol isomers, and the ketone group of isoguanine increases the basicity of the nitrogen at the 6-position. As we could not get a standard compound of 8-hydroxyadenine, it is still not clear what substance is observed in the chromatogram, but, on the basis of good coincidence with the retention time of isoguanine, it seems possible that isoguanine is formed from adenine. Therefore, adenine is converted into 2-hydroxyadenine and isomerized into isoguanine, which is considered to induced renal injury.

In conclusion, the basicity of nitrogen at the 6-position of purine ring is considered to be important in inducing renal injury. Furthermore, isoguanine has high affinity with kidney and also induces renal injury, and the renal injury induced by adenine is caused by isoguanine, although the mechanism remains unsolved.

REFERENCES

(1979).