Decreasing Effect of Allantoxanamide, a Hyperuricemic Agent on Renal Functions in Rats

Yukio YONETANI, Kazumi IWAKI and Yasunao OGAWA*
Shionogi Research Laboratories, Shionogi & Co., Ltd.,
Fukushima-ku, Osaka 553, Japan

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Abstract—Both potassium oxonate and allantoxanamide have been reported as useful reagents for inhibiting urate oxidase, with the hyperuricemic effect of allantoxanamide being longer-lasting than that of oxonate in rats. The present study was done to evaluate the utility of allantoxanamide for investigating problems concerning hyperuricemia using rats. A single intraperitoneal administration of 150 mg/kg allantoxanamide elevated the plasma uric acid level progressively during the experiment for 6 hr, resulting in a much higher level than that maintained by means of repeated dosing with 250 mg/kg potassium oxonate, i.p., at 2-hr intervals. Such a severe and long-lasting hyperuricemia caused by allantoxanamide was due to decreased renal function of uric acid excretion according to its nephrotoxicity in addition to the inhibition of urate oxidase like that by oxonate. Thus, we concluded that allantoxanamide might be a useful reagent for investigating the causes of hyperuricemia with renal failure, but was not a practical agent like oxonate in order to evaluate the response to uricosuric agents.

An apparent species difference in the metabolism of uric acid delayed studies on the problems concerning hyperuricemia, but the findings on practical urate oxidase inhibitors shed some light on various aspects of some of these problems in the pharmacological field using experimental animals. Johnson et al. (1, 2) found that oxonate and allantoxanamide are useful for producing model animals with hyperuricemia.

Recently, we devised a practical assay method for uricosuric activity using oxonate-treated rats (3), while no data has been reported on the utility of allantoxanamide for the evaluation of uricosuric agents. Thinking that the long-lasting hyperuricemic property of allantoxanamide might also be useful for producing model animals with inhibited urate oxidase, we conducted the present study to determine the characteristics of the agent from the viewpoint of its effects on renal function for uric acid excretion. Our results indicated that the long-lasting hyperuricemia caused by allantoxanamide is due to a reduction of renal excretion of uric acid in addition to the inhibition of urate oxidase, and therefore, the agent is not suitable for testing uricosuric drugs. On the other hand, it produced severe hyperuricemia with reduction of the glomerular filtration rate, proteinuria, and urinary occult blood, suggesting that the agent can be used for producing an experimental model for sustained hyperuricemia with associated nephropathy in rats.

Materials and Methods
Male Slc-Wistar strain rats of 9–10 weeks of age were used. To evaluate the hyperuricemic effect of the test agent, arterial catheters for blood collection and intraperitoneal tubes for drug administration were inserted into the animals according to the method described previously (4) at a week before the dosing. Potassium oxonate and allantoxanamide were purchased from

* Present address: Faculty of Pharmaceutical Sciences, Setsunan University, Osaka 573-01, Japan
Calbiochem, Co., and suspensions of these agents in 1% gum arabic solution were administered intraperitoneally at 2 ml/kg body weight. The animals were then housed in individual metabolic cages; potassium oxonate (250 mg/kg, i.p.) was given at 2-hr intervals, while allantoxanamide (150 mg/kg, i.p.) was administered only once at the beginning.

Blood was collected every 2 hr, and urine was collected after 6 hr. In the experiments using anesthetized rats to evaluate drug effects in nephrectomized animals, blood was collected from the jugular vein.

Clearance studies were carried out according to the method reported previously (3): the femoral artery, femoral vein and urinary bladder were cannulated for blood collection, infusion and urine collection, respectively. The animals were given 60% urethane, 2 ml/kg, s.c., and 15% inulin, 4 ml/kg, s.c., then an infusion of 4% mannitol-1.5% inulin-0.9% sodium chloride solution at the flow rate of 0.1 ml/min at 30°C.

After equilibration for 40 min, 0.2 ml of arterial blood was collected every 20 min, and five consecutive urine samples were collected at 20-min intervals. In testing drug effects on the uric acid clearance, test drugs were administered intraperitoneally just after the first urine collection. In the experiments to evaluate the effects of pretreated allantoxanamide, 150 mg/kg of the agent was intraperitoneally given 100 min prior to the beginning of infusion. Uric acid and allantoin were determined basically according to the methods of Sumi et al. (4–6), inulin was measured by the method of Vurek and Pegram (7), and urinary protein and occult blood were assessed using clinical test paper (LABSTIX-II, Miles Sankyo Co.). Data are shown as the mean value and standard error; the difference from the control level or the level before dosing was evaluated using Tukey's method after one-way analysis of variance.

**Results**

Hyperuricemic effects of potassium oxonate and allantoxanamide: As demonstrated

![Fig. 1. Effects of potassium oxonate and allantoxanamide on plasma uric acid and allantoin concentrations in rats. Results indicate the mean±standard error of 7–8 animals. Open circles, filled circles, open squares and filled squares are the mean values of the control, oxonate-treated, combination and allantoxanamide-treated groups, respectively. Bars are the standard error. Potassium oxonate (250 mg/kg, i.p.) was administered three times at 2 hr intervals, while allantoxanamide (150 mg/kg) was administered intraperitoneally once at the beginning. **: Significantly different from the value prior to the dosing at P < 0.05 and P < 0.01, respectively.](image)
by Johnson et al. (1, 2), both oxonate and allantoxanamide were hyperuricemic in rats. As shown in Fig. 1, a single administration of 150 mg/kg of allantoxanamide i.p. increased the plasma uric acid level progressively during the experiment for 6 hr with a slight rise of the allantoin concentration.

On the other hand, successive administrations of 250 mg/kg of potassium oxonate every 2 hr caused a hyperuricemic state with a reduction of the allantoin level; the plasma uric acid level, however, was conspicuously lower than that elicited by the single administration of allantoxanamide. Moreover, the combination dosing of both oxonate and allantoxanamide affected the uric acid and allantoin levels similarly to the administration of only oxonate. As represented in Table 1, the dosing of allantoxanamide increased renal weight and produced proteinuria and urinary occult blood. Although the urine-excreted amount of allantoin decreased, the amount of uric acid did not increase in spite of the severe hyperuricemia.

In contrast, the oxonate treatment was apparently diuretic, with an increase of uric acid excretion and a decrease of allantoin. Also the treatment produced no renal hypertrophy, proteinuria, or occult blood in comparison with the allantoxanamide treatment. The combination dosing was also diuretic and hyperuricosuric, causing no renal hypertrophy, but produced a mild proteinuria and some occult blood.

**Effects of nephrectomy on the treatments with oxonate and allantoxanamide:** Potassium oxonate, 250 mg/kg, and allantoxanamide, 150 mg/kg, were administered intraperitoneally just after nephrectomy under anesthesia with sodium pentobarbital; the plasma concentrations of uric acid and allantoin were examined 2 hr later. As shown in Fig. 2, both oxonate and allantoxanamide increased the uric acid level in the control experiment using rats with kidneys under a similar anesthetic condition, while a reduction of the allantoin level was observed only with the oxonate treatment. Therefore, the sum of uric acid and allantoin concentrations increased only with the allantoxanamide treatment. On the other hand, both the oxonate and allantoxanamide treatments similarly increased uric acid and reduced allantoin in nephrectomized rats which exhibited a

| Table 1. Effects of oxonate and allantoxanamide on renal weight and urinary components in rats |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Treatment                                       | No. of rats     | Renal weight (g/100g body weight) | Urinary protein | Urinary occult blood |
| Control                                         | 7               | 0.68±0.01       | - (7)           | - (7)              |
| Potassium oxonate                               | 7               | 0.68±0.01       | - (7)           | - (7)              |
| Allantoxanamide                                 | 8               | 1.06±0.01**     | ++ (1)          | ++ (1)             |
|                                                  |                 |                 | +++ (6)         | +++ (5)            |
| Potassium oxonate +                              | 7               | 0.70±0.01       | - (3)           | - (3)              |
| Allantoxanamide                                 |                 |                 | ++ (4)          | ++ (3)             |
|                                                  |                 |                 | + (1)           |                  |

| No. of rats | Urine volume (ml/kg-hr) | Urinary uric acid (mg/kg-hr) | Urinary allantoin (mg/kg-hr) |
|-------------|-------------------------|-------------------------------|-------------------------------|
| Control     | 7                       | 1.88±0.18                    | 0.40±0.05                     | 5.92±0.65                 |
| Potassium oxonate | 7   | 3.60±0.33**                | 3.67±0.62**                   | 1.88±0.15**               |
| Allantoxanamide | 8  | 1.02±0.15                  | 0.38±0.08                     | 1.28±0.22**               |
| Potassium oxonate + | 7  | 4.00±0.20**                | 3.10±0.25**                   | 2.38±0.17**               |

Data represent the mean±standard error for renal weight, urine volume, urinary uric acid and allantoin. The grades of urinary protein and occult blood were obtained using clinical test paper "LABSTIX-II"; the number of animals with the same grade is indicated in parentheses. Details on the dosing are described in the footnote of Fig. 1. **: Significantly different from the control level at P<0.01.
Fig. 2. Effects of nephrectomy on plasma uric acid and allantoin levels elicited by potassium oxonate and allantoxanamide in rats. Panel A shows the experimental results in rats with kidneys; panel B, those in nephrectomized rats. A filled column indicates the mean value of plasma uric acid level; an open column, that of allantoin. Bars are the standard error. Each group consisted of 4 animals. Column a shows the concentrations of uric acid and allantoin of the group treated with 1% gum arabic solution; column b, those of the 250 mg/kg potassium oxonate-treated group, and column c, those of the 150 mg/kg allantoxanamide-treated group. Both drugs were given intraperitoneally. **: Significantly different from the value of the group treated with gum arabic solution at P < 0.01.

marked increase of allantoin level.

Effects of osmotic diuresis with mannitol infusion on the treatments with allantoxanamide and oxonate: To evaluate the effect of osmotic diuresis with mannitol on the treatments with allantoxanamide and oxonate, clearance experiments were performed. As represented in Table 2, a single dose of 150 mg/kg of allantoxanamide i.p. which was administered 100 min prior to the beginning of infusion markedly reduced the glomerular filtration rate, resulting in the decrease of uric acid clearance.

The urine flow overshot the level of the control group during the infusion with mannitol, but the inulin clearance and uric acid clearance recovered only a little. The mannitol infusion increased the fractional excretion of uric acid (FEua) in allantoxanamide-treated animals more than that in the control group. However, the hyperuricosuric effect of mannitol infusion did not reduce the hyperuricemia elicited by allantoxanamide. Thus, although allantoxanamide was markedly hyperuricemic, the treatment varied overall function on uric acid excretion during the clearance experiments.

In contrast, the overall function in the kidneys of oxonate-treated rats was stable during the clearance experiments as described previously (3).

Next, the effects of allantoxanamide and oxonate were studied using animals with osmotic diuresis. As shown in Table 3, both 150 mg/kg of allantoxanamide and 250 mg/kg of potassium oxonate increased the plasma uric acid level, but the rise with the allantoxanamide treatment was rather less than that with the oxonate treatment. Both agents had almost no effect on renal functions for uric acid excretion. Thus, the hyperuricemic effect of allantoxanamide was not intrinsically more potent than that of oxonate in the rats with osmotic diuresis.

Discussion

According to Johnson et al. (1, 8), possible causes of the prolonged hyperuricemia with allantoxanamide are presumed to be extensive uric acid deposition in the kidney
Table 2. Effects of allantoxanamide on renal functions for uric acid excretion and plasma uric acid in rats treated prior to inducing osmotic diuresis

| Time (min) | Urine volume (ml/kg min) | Cin (ml/kg min) | Cua (ml/kg min) | FEua | Plasma uric acid (µg/ml) |
|------------|--------------------------|-----------------|-----------------|------|-------------------------|
| I. Control group (n=10) |
| -100: 1% Gum arabic solution, 2 ml/kg, i.p. |
| 0: Beginning of infusion with 4% mannitol-1.5% inulin-0.9% sodium chloride |
| 40–60 | 0.121±0.004 | 8.42±0.19 | 2.86±0.11 | 0.339±0.009 | 5.0±0.2 |
| 60–80 | 0.145±0.010 | 8.71±0.16 | 3.19±0.11 | 0.366±0.010 | 5.0±0.2 |
| 80–100 | 0.170±0.017 | 8.16±0.57 | 3.28±0.18 | 0.385±0.019 | 5.0±0.2 |
| 100–120 | 0.186±0.018 | 8.96±0.51 | 3.54±0.13 | 0.402±0.018 | 5.0±0.2 |
| 120–140 | 0.180±0.011 | 8.26±0.34 | 3.49±0.22 | 0.418±0.019 | 5.0±0.2 |
| II. Allantoxanamide-treated group (n=8) |
| -100: Allantoxanamide, 150 mg/kg, i.p. |
| 0: Beginning of infusion with 4% mannitol-1.5% inulin-0.9% sodium chloride |
| 40–60 | 0.069±0.012 | 0.67±0.17** | 0.21±0.04** | 0.277±0.022 | 48.1±1.6** |
| 60–80 | 0.183±0.026 | 2.04±0.22** | 0.70±0.09** | 0.342±0.022 | 51.3±1.8** |
| 80–100 | 0.236±0.027 | 2.09±0.21** | 0.98±0.12** | 0.472±0.028 | 53.2±1.9** |
| 100–120 | 0.288±0.027** | 2.26±0.15** | 1.25±0.14** | 0.548±0.032** | 55.6±2.1** |
| 120–140 | 0.327±0.027** | 2.34±0.20** | 1.46±0.20** | 0.608±0.037** | 60.0±2.0** |

Data represent the mean±standard error. The concentration of plasma uric acid in the control group was calculated as the average value of six samples from 40 to 140 min, because of the critically low level in the assay procedure. The numbers of animals are given in parentheses. Abbreviations are as follows: Cin, inulin clearance; Cua, uric acid clearance; and FEua, fractional excretion of uric acid. **: Significantly different from the value of the control group in the corresponding experimental period at P<0.01.

tubules due to severe hyperuricemia and the negative influence on de novo purine biosynthesis, compared with the positive effect of oxonate. However, the experimental results in the present study suggested that its renal effect was related to the severe and prolonged hyperuricemic effect. As seen in the experimental results using nephrectomized rats and rats with osmotic diuresis, the 150 mg/kg-allantoxanamide treatment was not always more hyperuricemic than the treatment with 250 mg/kg of potassium oxonate. Nevertheless, the allantoxanamide treatment in the animals without nephrectomy or osmotic diuresis produced a more severe and long-lasting hyperuricemia than the repeated dosing with 250 mg/kg of oxonate, together with a marked decrease of the glomerular filtration rate and renal hypertrophy with proteinuria and occult blood. These experimental results support that the decreased renal excretion of uric acid causes a severe and long-lasting hyperuricemia by allantoxanamide. If such nephrotoxic effects of allantoxanamide dissipated soon after the single administration, they might be missed in an investigation using this agent, unless experiments were performed to determine any functional changes in the kidneys after the dosing. Wexler (9) reported a hepatotoxic effect of allantoxanamide in rats which had been given 250 mg/kg of the agent i.p. three times weekly for 4 consecutive weeks, but noted that the kidneys appeared normal in the experiment. We also examined histologically the kidneys of rats which had received three consecutive daily dosing of 250 mg/kg of allantoxanamide i.p. Uric acid deposition in the renal tubules was clearly observed. The hyperuricemic effect with such uric acid deposition was lessened by a combination dosing with trichlormethiazide or furosemide, but a uricosuric drug with a mild diuretic effect, tienilic acid, was ineffective on the hyperuricemia. From these experimental
results, we concluded that allantoxanamide produced a severe and prolonged hyperuricemia due to its renal effect and inhibition of urate oxidase, and that the uric acid deposition in the renal tubules due to the severe hyperuricemia progressively aggravated the hyperuricemic effect. According to Johnson et al. (2), the hyperuricemic effect caused by treatment with 150 mg/kg allantoxanamide progressively increased for 6 hr and recovered within 24 hr, while a single 250 mg/kg dose of this drug gave rise to extensive uric acid deposition in the kidney tubules, resulting in an elevated blood level of uric acid 24 hr after dosage. In the present study, apparent reductions of glomerular filtration rate and uric acid clearance were observed from 140 to 240 min after the single dosing of 150 mg/kg, i.p., using a clearance technique (Table 2), so it appears that the reduction of glomerular filtration rate is related to the severe and long-lasting hyperuricemia elicited by allantoxanamide. However, such a reduction of glomerular filtration rate could not be recognized if the drug was given during the clearance experiments (Table 3). Therefore, it is not clear whether the reduction of glomerular filtration rate initiates the hyperuricemic effect or the uric acid deposition in the renal

Table 3. Effects of allantoxanamide and potassium oxonate on renal functions for uric acid excretion and plasma uric acid in rats with osmotic diuresis

| Time (min) | Urine volume (ml/kg·min) | Cin (ml/kg·min) | Cua (ml/kg·min) | FEua | Plasma uric acid (μg/ml) |
|-----------|--------------------------|-----------------|-----------------|------|-------------------------|
| Exp. I (n=7) | | | | | |
| 0: Beginning of infusion with 4% mannitol-1.5% inulin-0.9% sodium chloride | | | | | |
| 40–60 | 0.110±0.007 | 8.62±1.15 | 2.46±0.16 | 0.297±0.030 | 5.9±0.4 |
| 1% Gum arabic solution, 2 ml/kg i.p. | | | | | |
| 60–80 | 0.149±0.017 | 9.13±0.96 | 2.80±0.21 | 0.320±0.032 | 5.9±0.4 |
| 80–100 | 0.172±0.021* | 9.56±0.81 | 3.13±0.25 | 0.334±0.027 | 5.9±0.4 |
| 100–120 | 0.164±0.014** | 9.74±0.84 | 3.68±0.42 | 0.383±0.035 | 5.9±0.4 |
| 120–140 | 0.171±0.011** | 10.39±1.04 | 4.01±0.27** | 0.412±0.048 | 5.9±0.4 |
| Exp. II (n=6) | | | | | |
| 0: Beginning of infusion with 4% mannitol-1.5% inulin-0.9% sodium chloride | | | | | |
| 40–60 | 0.113±0.008 | 9.75±0.93 | 2.19±0.23 | 0.225±0.011 | 7.8±0.3 |
| Allantoxanamide, 150 mg/kg i.p. | | | | | |
| 60–80 | 0.146±0.006 | 11.00±0.76 | 2.48±0.20 | 0.228±0.016 | 11.3±0.5** |
| 80–100 | 0.148±0.006 | 10.92±0.64 | 1.88±0.23 | 0.176±0.026* | 15.6±0.6**++ |
| 100–120 | 0.153±0.006 | 10.51±0.59 | 2.54±0.23 | 0.241±0.015* | 17.8±0.7**++ |
| 120–140 | 0.158±0.007 | 11.34±0.95 | 3.40±0.36 | 0.301±0.024 | 19.3±0.8**++ |
| Exp. III (n=4) | | | | | |
| 0: Beginning of infusion with 4% mannitol-1.5% inulin-0.9% sodium chloride | | | | | |
| 40–60 | 0.104±0.004 | 9.10±1.08 | 1.90±0.16 | 0.213±0.019 | 8.4±0.4 |
| Potassium oxonate, 250 mg/kg i.p. | | | | | |
| 60–80 | 0.147±0.004 | 11.26±0.74 | 2.46±0.42 | 0.226±0.050 | 14.7±0.7**++ |
| 80–100 | 0.158±0.005 | 10.50±0.61 | 1.55±0.27 | 0.152±0.031* | 23.4±2.7**++ |
| 100–120 | 0.158±0.005 | 10.16±1.38 | 1.88±0.35 | 0.204±0.050 | 27.0±3.4**++ |
| 120–140 | 0.163±0.002 | 10.44±0.35 | 3.21±0.04 | 0.309±0.012 | 26.8±0.8**++ |

Data represent the mean±standard error. Plasma uric acid in Exp. I is shown as the average value of six samples from 40 to 140 min as described in Table 2. The numbers of animals are given in parentheses. *, **: Significantly different from the level before dosing at P<0.05 and P<0.01, respectively. *+; ++: Significantly different from the value in the corresponding experimental period of Exp. I at P<0.05 and P<0.01, respectively.
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tubules causes the reduction of glomerular filtration rate. The details of the effect of allantoxanamide on glomerular filtration rate should be further studied for evaluating the agent-treated rats as an experimental model for sustained hyperuricemia with associated nephropathy.

Although the detailed etiology of allantoxanamide-induced nephrotoxicity is not clear, the allantoxanamide blocking effect on uric acid excretion could be easily reduced by giving it in combination with oxonate, trichlormethiazide or furosemide.

Repeated administration of oxonate was diuretic and hyperuricosuric as had been already reported by Johnson et al. (1), although its single dosing was neither diuretic nor hyperuricosuric due to the effect on the tubular transport of uric acid. As seen in Table 1, the combination dosing of allantoxanamide with oxonate was definitely diuretic and hyperuricosuric like the treatment with oxonate alone, and it was less nephrotoxic than the dosing of allantoxanamide alone. Accordingly, although the detailed mechanism is not clear, it seems that the reduction of the hyperuricemic effect in the combination dosing from that by allantoxanamide alone is due to the diuretic and hyperuricosuric effects of oxonate. Thus, if allantoxanamide-treated rats are used as the animals with hyperuricemia, some diuretics would exhibit the therapeutic effect. However, the rats which had been given allantoxanamide were useless for evaluating the action of uricosuric agents, because of the marked reduction of the glomerular filtration rate caused by the pretreatment, and the variable overall function in the kidneys during the clearance experiments. The studies on the diuretics which are useful for improving the sustained hyperuricemia in allantoxanamide-treated rats as an experimental model with renal failure will be continued.

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