A New Diuretic That Does Not Reduce Renal Handling of Uric Acid in Rats, S-8666

Yukio YONETANI, Kazumi IWAKI, Mitsuo ISHII and Hiroshi HARADA
Shionogi Research Laboratories, Shionogi & Co., Ltd.,
Fukushima-ku, Osaka 553, Japan
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Abstract—The uric acid-retaining effects of diuretics were studied using sodium-restricted spontaneously hypertensive rats. Test agents were administered orally once a day for two weeks. Diuretic thiazides such as trichlormethiazide and hydrochlorothiazide and loop diuretics such as furosemide and indacrinone clearly reduced the renal function for uric acid excretion in treatment which produced major effects such as diuresis, saluresis and hypotension. However, a new diuretic with uricosuric activity, S-8666, developed in our laboratories, had no effect on the renal handling of uric acid at doses which showed major effects similar to those of other diuretics. The results should aid the understanding of the utility of S-8666 as a new diuretic antihypertensive which does not cause hyperuricemia during therapy.

The high incidence of hyperuricemia to hypertension (1, 2) has required the development of a new generation of diuretic antihypertensives, which do not cause hyperuricemia. Uricosuric diuretics such as tienilic acid (3) and indacrinone (4) have recently been studied as such candidates. However, the results have shown the need for careful testing (5, 6). In particular, the fact that potent activity for hyperuricosuria does not always produce an iso- or hypouricemic effect during use as medication, as found with indacrinone, has necessitated the more direct measurement of the hyperuricemic effect of diuretics. We have reported a practical procedure for determining decreased renal function on uric acid excretion following successive administration of diuretics to sodium-restricted spontaneously hypertensive rats (7). The procedure can be used to examine side effect signs of diuretic antihypertensives, although the hyperuricemic effect could not be evaluated because of the hepatic metabolism of uric acid in the animals.

As a candidate for a uricosuric diuretic, we suggested 5-dimethylsulfamoyl-6,7-dichloro-2,3-dihydrobenzofuran-2-carboxylic acid (S-8666), which is active in rats and chimpanzees (8). We tried to find whether S-8666 affects uric acid excretion at a dose which produces a major effect, in comparison with other diuretics. Our findings support the utility of S-8666 as a new diuretic antihypertensive which does not cause hyperuricemia.

Materials and Methods
Experiments were done using 15-16-week-old, CRJ-spontaneously hypertensive male rats (SHR) according to the method described previously (7); the animals were housed in individual metabolic cages and given a low sodium diet (Oriental Yeast Co., Tokyo) and tap water ad libitum. The diet was a solidified mixture of corn starch (38%), milk casein (25%), α-starch (10%), cellulose powder (8%), linoleic salad oil (6%), mineral mixture (6%), granulated sugar (5%) and a vitamin mixture (2%). The sodium and potassium contents were determined for every lot of the preparation by Japan Food Research Laboratories, Tokyo. The sodium content was 2-3 mEq/kg, while that of potassium was about 180 mEq/kg. The sodium and potassium contents in the drinking water were 0.7 and 0.07 mEq/l.
respectively.

Hydrochlorothiazide (Sigma) and furosemide (Sigma) were commercial preparations, while indacrinone and S-8666 were synthesized in our laboratories. These test compounds were suspended in 1% gum arabic solution and orally administered at 2 ml/kg of body weight once a day for 14 days from the start of the maintenance with a low sodium diet. Dosing of the test compounds was done at 5 PM every day, and the 24-hour urine between the daily administrations was collected from day 1 to 5 and from day 7 to 12. The amounts of daily intake of diet and water were also determined to calculate the intakes of sodium and potassium. Urinary concentration of sodium and potassium was determined by flame photometry using an Instrumentation Laboratory Model 943, Italy. The systolic blood pressure of the animals was measured indirectly using a tail pulse manometer (Natsume, Model KN-210, Tokyo) without anesthesia on day 0, 6 and 13 between 10 AM and 3 PM. On day 14, a clearance study was done to determine the effect on renal handling of uric acid under anesthesia with pentobarbital by the method described previously (9). The clearance experiment was performed three times at 20-min intervals, then the average values were found. To measure the hematocrit value and plasma electrolyte concentrations, arterial blood (0.3 ml) was sampled just after the cannulation for the clearance experiment.

Uric acid was fluorometrically determined basically according to the method of Sumi et al. (10), and inulin was estimated by the method of Vurek and Pegram (11).

The abbreviations used here are: Cin, inulin clearance; Cua, uric acid clearance; FEua, fractional excretion of uric acid (Cua/Cin). Data are indicated as the mean±standard error, and the statistical significance of the difference from the control level was evaluated by Student’s t-test.

**Results**

Every compound was tested at doses which produced appreciable major effects such as diuresis, natriuresis and hypotension. Sodium excretion of the animals decreased progressively with maintenance on a low sodium diet, then reached at a stable low level a few days later. The diuretic and

| Treatment          | Rats | Urine volume (ml/kg) | Urinary sodium (mEq/kg) | Urinary potassium (mEq/kg) |
|--------------------|------|----------------------|-------------------------|---------------------------|
| Control            | 8    | 174±11               | 2.89±0.24               | 37.0±1.4                  |
| Hydrochlorothiazide| 10 mg/kg | 8       | 339±44**               | 7.27±0.11**              | 37.4±1.7                  |
|                    | 20 mg/kg | 8       | 435±59**               | 6.78±0.11**              | 36.1±2.3                  |
|                    | 50 mg/kg | 8       | 403±34**               | 6.44±0.23**              | 35.0±1.9                  |
| Control            | 8    | 199±17               | 2.91±0.19               | 38.2±0.9                  |
| Furosemide         | 10 mg/kg | 7       | 182±13                 | 2.97±0.28                | 38.4±0.6                  |
|                    | 20 mg/kg | 7       | 258±13*                | 6.19±0.35**              | 33.5±3.1                  |
| Control            | 8    | 186±19               | 2.21±0.23               | 40.0±1.2                  |
| Indacrinone        | 20 mg/kg | 7       | 164±14                 | 2.66±0.20                | 33.7±1.7**                |
|                    | 50 mg/kg | 7       | 210±11                 | 3.64±0.34**              | 34.0±1.8*                 |
| Control            | 8    | 198±13               | 3.43±0.27               | 41.0±2.0                  |
| S-8666             | 100 mg/kg | 8      | 310±18**               | 6.77±0.30**              | 37.9±1.2                  |

Urine volume and urine-excreted amounts of sodium and potassium are shown as the sums for 5 days, and the data represent the mean±standard error. Test compounds were administered orally once a day for 14 days. **: Significantly different from the control level at P<0.05 and 0.01, respectively.
Table 2. Diuretic and saluretic effects of diuretic antihypertensives in sodium-restricted SHR (from day 7 to 12)

| Treatment                  | Rats | Urine volume (ml/kg) | Urinary sodium (mEq/kg) | Urinary potassium (mEq/kg) |
|----------------------------|------|----------------------|-------------------------|---------------------------|
| Control                    | 8    | 314±26               | 0.33±0.05               | 51.1±1.2                  |
| Hydrochlorothiazide        |      |                      |                         |                           |
| 10 mg/kg                   | 8    | 564±67**             | 1.50±0.14**             | 49.8±1.0                  |
| 20 mg/kg                   | 8    | 732±102**            | 0.75±0.16*              | 50.8±2.0                  |
| 50 mg/kg                   | 8    | 734±44**             | 0.88±0.12**             | 53.4±1.4                  |
| Control                    | 8    | 268±14               | 0.32±0.04               | 34.0±1.1                  |
| Furosemide                 |      |                      |                         |                           |
| 10 mg/kg                   | 7    | 227±15               | 0.64±0.10**             | 35.4±1.0                  |
| 20 mg/kg                   | 7    | 319±28               | 2.24±0.44**             | 32.3±1.7                  |
| Control                    | 8    | 268±33               | 0.28±0.03               | 43.7±1.1                  |
| Indacrinone                |      |                      |                         |                           |
| 20 mg/kg                   | 7    | 300±19               | 0.61±0.11**             | 43.6±1.5                  |
| 50 mg/kg                   | 7    | 510±42**             | 1.69±0.21**             | 42.7±1.4                  |
| Control                    | 8    | 313±20               | 0.32±0.05               | 46.8±1.9                  |
| S-8666                     |      |                      |                         |                           |
| 100 mg/kg                  | 8    | 445±16**             | 1.91±0.20**             | 52.4±1.2*                 |

Urine volume and urine-excreted amounts of sodium and potassium are the sums for 6 days, and the data represent the mean ± standard error. Treatments of test drugs are described in Table 1. * **: Significantly different from the control level at P<0.05 and 0.01, respectively.

Saluretic effects of the test compounds were estimated for the two periods in which the basal levels of sodium excretion were obviously different from each other. As indicated in Tables 1 and 2, the urine volume in the first week was less than that in the second week in the control group, while urine-excreted amounts of sodium in the first week were much more than that during the second week. Urinary excretion of potassium was stable during the experimental period.

Hydrochlorothiazide was markedly diuretic and natriuretic during both weeks at daily doses of more than 10 mg/kg, although the effects were not dose-dependent. Furosemide was less diuretic, but natriuretic at 20 mg/kg, like hydrochlorothiazide.

Doses of more than 20 mg/kg of furosemide produced more marked diuresis, and the experiments had to be stopped due to a marked reduction of food intake by the animals. Indacrinone was also less diuretic and natriuretic in the animals, but 50 mg/kg was natriuretic and particularly effective in the second week. This compound also reduced the food intake at high doses. A mild decrease of urinary potassium at the early stage was related to the reduction of food intake, but recovered during the consecutive administration at the doses of 20 and 50 mg/kg. On the other hand, S-8666 had no effect on the food intake at the high doses producing marked diuresis and natriuresis, as found with hydrochlorothiazide. All test compounds were hypotensive at the natriuretic doses, as shown in Table 3.

The hypotensive effect appeared during the first week and continued to decrease in the next week.

Some side effect signs were found after successive drug administration for 14 days. All test compounds slightly increased the hematocrit value at the hypotensive doses, as shown in Table 4. Hydrochlorothiazide and furosemide reduced the plasma sodium level, while hydrochlorothiazide and 50 mg/kg of indacrinone caused hypokalemia. S-8666, however, caused no decrease of plasma sodium and potassium; and in fact, the plasma sodium level increased in spite of the drug being highly natriuretic like hydrochlorothiazide and furosemide.

As seen in Table 5, hydrochlorothiazide,
Furosemide and indacrinone seemed to reduce the renal handling of uric acid, while S-8666 had no effect on the renal function. In the clearance studies, hydrochlorothiazide and indacrinone decreased both glomerular filtration and tubular transport of uric acid, while furosemide reduced only the tubular transport system, and S-8666 had no effect.

### Table 3. Hypotensive effects of diuretic antihypertensives in sodium-restricted SHR

| Treatment               | Rats | Systolic blood pressure (mmHg) |
|-------------------------|------|--------------------------------|
|                         |      | Day 0 | Day 6 | Day 13 |
| Control                 | 8    | 197±2 | 186±4 | 187±3  |
| Hydrochlorothiazide     |      |       |       |        |
| 10 mg/kg                | 8    | 196±2 | 171±2*| 178±2* |
| 20 mg/kg                | 8    | 195±1 | 168±2*| 172±2* |
| 50 mg/kg                | 8    | 198±1 | 165±3*| 171±2* |
| Control                 | 8    | 212±4 | 193±3 | 189±2  |
| Furosemide              |      |       |       |        |
| 10 mg/kg                | 7    | 210±4 | 189±2 | 191±4  |
| 20 mg/kg                | 7    | 213±3 | 177±3*| 177±4* |
| Control                 | 8    | 215±3 | 196±3 | 199±2  |
| Indacrinone             |      |       |       |        |
| 20 mg/kg                | 7    | 219±4 | 182±3*| 183±4* |
| 50 mg/kg                | 7    | 214±4 | 171±3*| 166±3* |
| Control                 | 8    | 203±4 | 196±3 | 191±3  |
| S-8666                  |      |       |       |        |
| 100 mg/kg               | 8    | 205±3 | 188±3*| 176±4* |

Treatments of test drugs are described in Table 1. Data represent the mean±standard error. *,**: Significantly different from the control level at P<0.05 and 0.01, respectively.

### Table 4. Effects of diuretic antihypertensives on hematocrit value, plasma sodium and potassium concentrations in sodium-restricted SHR

| Treatment               | Rats | Hematocrit (%) | Plasma sodium (mEq/l) | Plasma potassium (mEq/l) |
|-------------------------|------|----------------|-----------------------|--------------------------|
|                         |      |                |                       |                          |
| Control                 | 8    | 49.1±0.4       | 144.4±0.4             | 3.65±0.06                |
| Hydrochlorothiazide     |      |                |                       |                          |
| 10 mg/kg                | 8    | 51.8±0.7**     | 144.1±0.8             | 3.82±0.12                |
| 20 mg/kg                | 8    | 51.5±0.5**     | 142.7±0.2**           | 3.25±0.07**              |
| 50 mg/kg                | 8    | 52.8±0.7**     | 142.8±0.3**           | 3.27±0.07**              |
| Control                 | 8    | 49.6±0.5       | 138.2±0.8             | 3.80±0.15                |
| Furosemide              |      |                |                       |                          |
| 10 mg/kg                | 7    | 49.2±0.3       | 136.9±0.4             | 3.78±0.12                |
| 20 mg/kg                | 7    | 52.0±0.8*      | 134.8±0.9*            | 3.70±0.12                |
| Control                 | 8    | 48.4±0.4       | 139.2±1.0             | 3.48±0.06                |
| Indacrinone             |      |                |                       |                          |
| 20 mg/kg                | 7    | 49.8±0.4*      | 139.0±0.4             | 3.46±0.07                |
| 50 mg/kg                | 7    | 50.9±0.5**     | 137.7±1.2             | 3.22±0.07*               |
| Control                 | 8    | 48.6±0.3       | 144.0±0.5             | 3.67±0.08                |
| S-8666                  |      |                |                       |                          |
| 100 mg/kg               | 8    | 51.3±0.7**     | 147.1±0.4**           | 3.70±0.09                |

Blood collection was done after the consecutive dosing for 14 days. Treatments of test drugs are described in Table 1, and data represent the mean±standard error. *,**: Significantly different from the control level at P<0.05 and 0.01, respectively.
Table 5. Effects of diuretic antihypertensives on the renal handling of uric acid in sodium-restricted SHR

| Treatment         | Rats | Cin (ml/kg·min) | Cua (ml/kg·min) | FEua   |
|-------------------|------|-----------------|-----------------|--------|
| Control           | 8    | 9.07±0.29       | 2.97±0.15       | 0.33±0.02 |
| Hydrochlorothiazide 10 mg/kg | 8    | 7.83±0.54       | 2.12±0.32*      | 0.26±0.03* |
|                   | 20 mg/kg | 8   | 7.15±0.83*      | 2.02±0.38**   | 0.27±0.02*  |
|                   | 50 mg/kg | 8   | 5.60±0.45**     | 1.38±0.20**   | 0.24±0.02** |
| Control           | 8    | 10.43±0.47      | 3.65±0.16       | 0.35±0.01 |
| Furosemide 10 mg/kg | 7    | 9.28±0.88       | 2.70±0.23**     | 0.30±0.01** |
|                   | 20 mg/kg | 7   | 8.99±0.85       | 2.85±0.30*    | 0.32±0.01*  |
| Control           | 8    | 9.08±0.41       | 3.49±0.27       | 0.38±0.02 |
| Indacrinone 20 mg/kg | 7    | 8.79±0.49       | 2.85±0.26       | 0.32±0.02* |
|                   | 50 mg/kg | 7   | 5.01±0.73**     | 1.50±0.27**   | 0.29±0.02** |
| Control           | 8    | 8.02±0.91       | 3.36±0.22       | 0.44±0.04 |
| S-8666 100 mg/kg  | 8    | 8.40±0.57       | 3.17±0.15       | 0.39±0.01 |

Clearance experiments were performed after the consecutive dosing for 14 days. Treatments of test drugs are described in Table 1, and data represent the mean±standard error. *,**: Significantly different from the control level at P<0.05 and 0.01, respectively.

Discussion

The new drug candidate S-8666, chosen by our unique method using oxonate-treated rats, was confirmed to be uricosuric and diuretic in chimpanzees (8). It was also less toxic than tienilic acid and indacrinone in various experimental animals and more antihypertensive than tienilic acid in the hypertensive rats established with deoxycorticosterone acetate and sodium chloride (Y. Yonetani et al., data unpublished). Therefore, unless its diuretic character produces a marked reduction of uric acid excretion, as has been found with indacrinone, S-8666 may serve as a practical uricosuric diuretic.

The present study supported our expectation in sodium-restricted SHR. Generally, rats have a hepatic function for metabolizing uric acid to allantoin, and their renal function for excreting uric acid is not easily affected by the oral administration of uricosuric drugs. However, though the consecutive dosing of diuretic thiazides was less diuretic and did not so easily reduce the renal function on uric acid excretion in the animals fed an ordinary diet, as generally accepted in humans, the decreased renal function on uric acid excretion following the diuretic administration apparently appeared under feeding with a low sodium diet (12). SHR fed the same low sodium diet appreciably decreased their blood pressure with the consecutive administration of trichlormethiazide (7). As presented in this report, similar reduction of the function on uric acid excretion is also recognized by hydrochlorothiazide, furosemide and even indacrinone. All of these known diuretic antihypertensives have been well known to be hyperuricemic during their use as medication in humans, independently of being uricosuric as proven with indacrinone. The reduction of the renal function on uric acid excretion is not always connected with a hyperuricemic effect in the rats having hepatic metabolism of uric acid, but probably as the cause of hyperuricemia in humans lacking the hepatic function.

The reduction of renal function on uric acid excretion in rats following the administration of diuretic thiazides might be brought about by a functional change of the kidneys dependent upon the marked diuresis which produced the volume loss in the animals, as...
described previously (9). However, furosemide and indacrinone also decreased the renal function on uric acid excretion in spite of being obviously less diuretic and natriuretic throughout the experimental period than in the cases of diuretic thiazides. Thus, mechanisms for the reduced renal excretion of uric acid following administration of various diuretics may not always be identical.

The dosing of diuretics was started simultaneously with feeding of a low sodium diet in the present experiments. Such a procedure is needed to evaluate the antihypertensive effect of the tested diuretics. As generally accepted, the blood pressure of SHR is insensitive to the treatment with diuretics when they are fed an ordinary diet. When the dosing of diuretics was carried out after equilibration to a low sodium diet for several days, test agents had no effect on the blood pressure, though being also diuretic and natriuretic (data not shown).

However, the systolic blood pressure apparently decreased in the diuretic administration to the SHR, simultaneously when a low sodium diet was given. Thus, the hypotensive effect of test diuretics in the present study was discovered in the first week of the dosing, and no further decrease of blood pressure was found in the next week, even though marked diuresis and natriuresis occurred.

Thus, the experimental procedure applied here can be used for evaluating simultaneously both the major and side effects of diuretic antihypertensives. The fact that S-8666 had no effect on the renal function for excreting uric acid in the experiment supported the utility of the new candidate as a different type of diuretic antihypertensive. The different character of S-8666 can be better understood by taking into consideration the difference in the potency and efficacy among diuretics. As generally accepted, diuretic thiazides are low-ceilinged diuretics, and hence a long-lasting effect using an excessive dose is necessary to produce their antihypertensive efficacy. On the other hand, as demonstrated previously using oxonate-treated rats (8), S-8666 is a high-ceilinged diuretic like furosemide. Accordingly, although the potency of its diuretic and natriuretic effects is low, the efficacy with a high dose is so high that the antihypertensive effect may be expected without a prolonged diuresis. The diuretic and natriuretic effects of test drugs were assessed using 24-hr urine samples in the present experiments. However, as the diuretic effect of S-8666, 100 mg/kg, p.o., in the rats usually was complete within several hours after the dosing under various experimental conditions (data not shown), we assume that the diuretic effect of S-8666 is not very long-lasting. The duration on the diuretic effect of test drugs should be further studied in order to understand the different characteristics of uric acid excretion between S-8666 and diuretic thiazides.

Next, the differences among S-8666, furosemide and indacrinone need to be discussed. Both furosemide and indacrinone decreased the food intake of the animals at relatively low doses, forcing termination of the experiments with the high doses. S-8666, however, had no effect on the food intake even at a high dose which was markedly diuretic and natriuretic like hydrochlorothiazide. The uricosuric activity of indacrinone has also been demonstrated in rats (13), but it could not normalize the renal function on uric acid excretion in sodium-restricted SHR. S-8666, however, allowed normal renal handling of uric acid in spite of being more diuretic than furosemide and indacrinone. Therefore, although the different character of S-8666 on the uric acid excretion from that of furosemide may be dependent upon its uricosuric activity, another possibility is that the temporarily uricosuric activity is independent of preventing the decrease of renal function on uric acid excretion in the consecutive dosing. Thus, even though furosemide and indacrinone decrease the renal excretion of uric acid, independent of their diuretic action, S-8666 would not cause such a renal effect.

S-8666 caused no hypokalemia in the present study, unlike hydrochlorothiazide and indacrinone. However, this favorable characteristic may be dependent upon the experimental conditions, such as a special
diet. In fact, we have found that all the tested drugs were hypokalemic in the hypertensive rats which were established with deoxycorticosterone acetate and sodium chloride. As we have seen, S-8666 differs from other diuretics in its influence on uric acid excretion. Further tests are needed for this new class of diuretic antihypertensive which does not cause uric acid retention.

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