Full Paper

The Diuretic Effect of *Sairei-to* Is Mediated by Nitric Oxide Production in Pentobarbital-Anesthetized Rats

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Abstract. *Sairei-to* (TJ-114), a Japanese traditional medicine, has been used clinically for the treatment of various edematous disorders. The inhibitory effect on edema may be dependent on the diuretic response to TJ-114. This study was conducted to clarify the mechanism of diuresis. Pentobarbital-anesthetized rats were infused with a saline solution intravenously (0.4 mL/30 min). Urine was collected through a bladder cannula for 30 min. Intraduodenal administration of TJ-114 (0.5–1.5 g/kg) resulted in a dose-dependent increase of urine volume with insignificant urinary sodium excretion and significant urea excretion, but no effect on mean arterial blood pressure. Furthermore, TJ-114 significantly increased urinary levels of NO₂ + NO₃. In addition, intraperitoneal pre-treatment with 6 mg/kg of N^G-nitro-L-arginine methyl ester inhibited the increase in urine volume, urinary urea excretion, and urinary levels of NO₂ + NO₃ in the rats treated with TJ-114. These results suggest that TJ-114 induces a diuretic response via production of NO.

Keywords: *Sairei-to*, nitric oxide, diuresis, edema

Introduction

The Japanese traditional medicine “*Sairei-to* (TJ-114)” has been used empirically for some time in the treatment of various edematous disorders (nephrotic syndrome, cirrhosis, pregnancy, swelling, and lymphedema after surgery and macular edema (1, 2)). In general, it is considered that an enhanced diuresis is useful for the treatment of edema with characteristics of sodium and water retention in the body. The beneficial effect on edema may be dependent on the diuretic response to TJ-114. It has been reported that TJ-114 increased urine volume in rats (3) and mice (4), but, the scientific evidence still remains insufficient. Thus, we investigated the mechanisms behind the diuretic response to TJ-114 in pentobarbital-anesthetized rats with water retention in the body. Under pentobarbital anesthesia, the stimulation of vasopressin release and activation of the sympathetic nervous system and renin-angiotensin system have been known to occur, with a resulting decrease in urine volume (5). We expected new

evidence supporting the diuretic effect of TJ-114 to come from studies using this animal model.

Materials and Methods

Animals and drugs

Male Wistar rats were obtained from SLC (Hamamatsu). They were housed in a cage and allowed free access to water and standard laboratory food (MF; Oriental Yeast, Tokyo). All experimental procedures were performed according to the “Guidelines for the Care and Use of Laboratory Animals” approved by the Laboratory Animal Committee of Tsumura & Co.

Tsumura *Sairei-to* (TJ-114) was prepared as a spray-dried powder from a hot-water extract and is composed of 12 crude drugs in fixed proportions: 7.0 g *Bupleuri radix*, 5.0 g *Pinelliae tuber*, 4.0 g *Alismatis rhizoma*, 3.0 g *Scutellariae radix*, 3.0 g *Ginseng radix*, 3.0 g *Zizyphi fructus*, 3.0 g *Polyporus*, 3.0 g *Atractylodis lancea rhizoma*, 3.0 g *Hoelen*, 2.0 g *Glycyrrhizae radix*, 1.5 g *Cinnamomi cortex*, and 1.0 g *Zingiberis siccaturum rhizoma*. N^G-Nitro-L-arginine methyl ester (L-NAME), and furosemide were purchased from Sigma (St. Louis, MO, USA).

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Operations

Rats (230 to 250 g) fasted overnight were anesthetized by intraperitoneal injection of sodium pentobarbital, 50 mg/kg body weight. Following a tracheotomy, the left jugular vein and carotid artery were cannulated with polyethylene tubing (PE 50), the latter for continuous monitoring of mean arterial blood pressure (MABP), the former for infusion of a saline solution with a priming volume of 0.8 mL, delivered at a rate of 0.4 mL/30 min. The bladder was cannulated through a small abdominal incision for collecting urine. The duodenum was cannulated for the administration of TJ-114 or furosemide. The rats were positioned on a heated table (37°C) and left for 1 h.

Experimental procedure

Experiment 1: TJ-114 (0.5, 1.0, or 1.5 g/kg) prepared in distilled water and the vehicle (5 mL/kg distilled water) were administered to rats ($n = 6 - 7$). Urine was collected for a period of 30 min before and at 30 and 60 min after the administration of TJ-114.

Experiment 2: The influence of L-NAME, a nitric oxide (NO) synthase inhibitor, on the effect of TJ-114 (1.5 g/kg) or vehicle-treated rats ($n = 6$ each) was examined. L-NAME (6 mg/kg, i.p.) was injected 5 min before the administration of drug.

Experiment 3: As a positive control, the loop diuretic furosemide (12 mg/kg) with or without L-NAME and the vehicle (0.5% Tween-80) were administered to rats ($n = 4 - 6$).

Measurements

MABP was measured using a pressure transducer. Urine sodium and potassium concentrations were measured by flame photometry (model 710 flame photometer; Hitachi, Tokyo) and urinary $\text{NO}_2 + \text{NO}_3$ with the Griess assay (6). Urine osmolality was determined by freezing point depression (3D3 osmometer; Advanced Instruments, Needham Height, MA, USA).

The urinary urea nitrogen concentration was determined by subtracting the ammonium nitrogen concentration using the urease-indophenol method (Urea nitrogen B-test; Wako, Tokyo).

Calculation

Electrolyte-free water reabsorption ($\text{E-Tc}_{\text{H}_2\text{O}}$) and electrolyte clearance (E-Cosm) were calculated. The formulae are as follows:

$$\text{E-Tc}_{\text{H}_2\text{O}} = \text{E-Cosm} - \text{UV}$$

$$\text{E-Cosm} = [(U_{\text{Na}} + U_{\text{K}}) \text{UV}] / (P_{\text{Na}} + P_{\text{K}})$$

UV is urine volume, U_{Na} is urinary sodium concentration, U_{K} is urinary potassium concentration, P_{Na} is plasma sodium concentration, and P_{K} is plasma potassium concentration.

We calculated E-Cosm assuming that P_{Na} plus P_{K} was constant at 150 mEq/L. In addition, for the determination of osmolar clearance (Cosm) and free water reabsorption ($\text{Tc}_{\text{H}_2\text{O}}$), 300 mOsm was used for plasma osmolality.

Statistical analyses

Data were expressed as the mean $\pm$ S.E.M. The statistical differences between groups were evaluated using Student's *t*-test or a one-way analysis of variance (ANOVA) followed by Dunnett's test or the Bonferroni test. Differences were considered to be statistically significant if $P < 0.05$.

Results

Urine volume and urinary parameters

As shown in Table 1, no significant differences in basal urine volume and urinary parameters before the TJ-114 or furosemide administration in pentobarbital-anesthetized rats were observed in any group.

TJ-114 dose-dependently increased urine volume in pentobarbital-anesthetized rats. A similar result with TJ-114 (1.5 g/kg) was observed in the furosemide

Table 1. Basal levels of urine parameters in rats anesthetized with pentobarbital

	n	Urine volume (mL/30 min)	Sodium ($\mu\text{Eq}/30 \text{ min}$)	Potassium ($\mu\text{Eq}/30 \text{ min}$)	Urea nitrogen (mg/30 min)	Osmolality (osm/kg H_2O)
Vehicle (distilled water)	6	0.107 ± 0.006	5.33 ± 1.92	23.1 ± 1.9	2.51 ± 0.42	1778 ± 113
TJ-114, 0.5 g/kg	6	0.102 ± 0.004	3.68 ± 0.88	21.3 ± 1.5	2.68 ± 0.34	1848 ± 100
TJ-114, 1.0 g/kg	6	0.109 ± 0.006	2.77 ± 0.34	25.5 ± 2.4	3.14 ± 0.43	2000 ± 87
TJ-114, 1.5 g/kg	7	0.103 ± 0.005	3.81 ± 0.57	22.9 ± 1.0	2.19 ± 0.18	1754 ± 34
Vehicle (0.5% Tween-80)	6	0.100 ± 0.009	3.36 ± 0.94	22.4 ± 3.4	3.60 ± 0.31	2253 ± 52
Furosemide	6	0.100 ± 0.006	3.72 ± 0.89	20.3 ± 2.3	3.21 ± 0.41	2070 ± 116

Each value is expressed as a mean $\pm$ S.E.M. No significant differences between drug-treated groups and the vehicle-treated group with one-way ANOVA followed by Dunnett's test or Student's *t*-test.

(12 mg/kg)-treated group (Fig. 1). As shown in Table 2, urinary sodium excretion was insignificantly increased 1.8-fold compared to the initial basal value 30–60 min after administration of TJ-114, although it was increased about fivefold after administration of furosemide. Urinary potassium excretion was modestly increased by treatment with TJ-114 and furosemide, in comparison with the corresponding control in each group. Urinary urea nitrogen excretion was significantly increased

by treatment with 1.5 g/kg of TJ-114, but not changed by treatment with furosemide. Urine osmolality was significantly decreased by treatment with TJ-114 in a dose-dependent manner. However, changes were minute compared with the effect of furosemide. As shown in Table 3, TJ-114 significantly increased Cosm , $\text{Tc}_{\text{H}_2\text{O}}$, and E-Cosm , but it did not change $\text{E-Tc}_{\text{H}_2\text{O}}$. Furosemide elevated both Cosm and E-Cosm (Table 4).

Fig. 1. Effect of TJ-114 and furosemide on urine volume in pentobarbital-anesthetized rats. ** $P < 0.01$, compared to Vehicle (distilled water), one-way ANOVA followed by Dunnett's test ($n = 6$ or 7); # $P < 0.05$, compared to Vehicle (0.5% Tween-80), Student's t -test ($n = 6$).

Table 2. Effects of TJ-114 and furosemide on the urine parameters in pentobarbital-anesthetized rats

Time after treatment	n	Urinary sodium excretion (%)		Urinary potassium excretion (%)		Urinary urea nitrogen (%)		Urine osmolality (%)	
		0–30 min	30–60 min	0–30 min	30–60 min	0–30 min	30–60 min	0–30 min	30–60 min
Vehicle (distilled water)	6	97 ± 13	111 ± 24	93 ± 4	124 ± 12	129 ± 8	179 ± 21	107 ± 3	113 ± 5
TJ-114, 0.5 g/kg	6	126 ± 5	169 ± 27	139 ± 7**	159 ± 17	157 ± 10	196 ± 26	105 ± 3	111 ± 4
TJ-114, 1.0 g/kg	6	143 ± 25	180 ± 44	147 ± 9**	161 ± 12	152 ± 8	172 ± 25	94 ± 2**	99 ± 1*
TJ-114, 1.5 g/kg	7	143 ± 17	181 ± 30	135 ± 10**	182 ± 19*	170 ± 14*	237 ± 25	92 ± 2**	84 ± 3**
Vehicle (0.5% Tween-80)	6	99 ± 17	122 ± 32	81 ± 8	79 ± 11	121 ± 7	139 ± 12	109 ± 3	108 ± 5
Furosemide	6	232 ± 40#	514 ± 64##	132 ± 12##	226 ± 17##	150 ± 18	154 ± 18	68 ± 6##	58 ± 6##

% of basal value is expressed as a mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$, compared to Vehicle (distilled water), with one-way ANOVA followed by Dunnett's test; # $P < 0.05$, ## $P < 0.01$, compared to Vehicle (0.5% Tween-80), with Student's t -test.

Table 3. Effects of TJ-114 on Cosm, Tc_{H₂O}, E-Cosm, and E-Tc_{H₂O} in pentobarbital-anesthetized rats

Time after treatment		Basal	0 – 30 min	30 – 60 min
Cosm (ml/30 min)	Vehicle	0.64 ± 0.07	0.72 ± 0.06	0.90 ± 0.04
	TJ-114, 0.5 g/kg	0.63 ± 0.05	0.90 ± 0.05	1.03 ± 0.08
	TJ-114, 1.0 g/kg	0.74 ± 0.07	1.05 ± 0.07**	1.12 ± 0.05
	TJ-114, 1.5 g/kg	0.60 ± 0.03	0.95 ± 0.06*	1.41 ± 0.13**
Tc _{H₂O} (ml/30 min)	Vehicle	0.53 ± 0.06	0.61 ± 0.06	0.77 ± 0.04
	TJ-114, 0.5 g/kg	0.53 ± 0.04	0.76 ± 0.05	0.87 ± 0.07
	TJ-114, 1.0 g/kg	0.63 ± 0.06	0.88 ± 0.06**	0.95 ± 0.05
	TJ-114, 1.5 g/kg	0.50 ± 0.02	0.77 ± 0.04	1.12 ± 0.09**
E-Cosm (ml/30 min)	Vehicle	0.19 ± 0.02	0.17 ± 0.02	0.22 ± 0.02
	TJ-114, 0.5 g/kg	0.17 ± 0.01	0.23 ± 0.02	0.26 ± 0.03
	TJ-114, 1.0 g/kg	0.19 ± 0.02	0.27 ± 0.02**	0.30 ± 0.02
	TJ-114, 1.5 g/kg	0.18 ± 0.01	0.24 ± 0.02	0.32 ± 0.03*
E-Tc _{H₂O} (ml/30 min)	Vehicle	0.083 ± 0.019	0.056 ± 0.016	0.080 ± 0.018
	TJ-114, 0.5 g/kg	0.065 ± 0.012	0.087 ± 0.013	0.110 ± 0.022
	TJ-114, 1.0 g/kg	0.079 ± 0.013	0.104 ± 0.020	0.127 ± 0.017
	TJ-114, 1.5 g/kg	0.076 ± 0.006	0.060 ± 0.016	0.020 ± 0.049

Each value is expressed as a mean ± S.E.M. **P*<0.05, ***P*<0.01 compared to Vehicle with one-way ANOVA followed by Dunnett's test.

Table 4. Effects of furosemide on Cosm, Tc_{H₂O}, E-Cosm, and E-Tc_{H₂O} in pentobarbital-anesthetized rats

Time after treatment		Basal	0 – 30 min	30 – 60 min
Cosm (ml/30 min)	Vehicle	0.75 ± 0.06	0.84 ± 0.07	0.90 ± 0.06
	Furosemide	0.70 ± 0.07	0.94 ± 0.06	1.20 ± 0.08*
Tc _{H₂O} (ml/30 min)	Vehicle	0.65 ± 0.05	0.74 ± 0.06	0.79 ± 0.05
	Furosemide	0.60 ± 0.07	0.72 ± 0.04	0.87 ± 0.07
E-Cosm (ml/30 min)	Vehicle	0.17 ± 0.03	0.14 ± 0.03	0.13 ± 0.02
	Furosemide	0.16 ± 0.02	0.23 ± 0.04	0.43 ± 0.06**
E-Tc _{H₂O} (ml/30 min)	Vehicle	0.072 ± 0.021	0.038 ± 0.021	0.023 ± 0.017
	Furosemide	0.060 ± 0.016	0.018 ± 0.016	0.100 ± 0.038

Each value is expressed as a mean ± S.E.M. **P*<0.05, ***P*<0.01 compared to Vehicle with Student's *t*-test.

The influence of L-NAME on the diuretic effect of TJ-114 or furosemide

In the experiments on the influence of L-NAME on the diuretic effect of TJ-114 or furosemide, the rate of basal urinary NO₂ + NO₃ excretion was 2.9 ± 0.5 nmol/min in the control group, and the basal value did not differ significantly in any group. As shown in Fig. 2, enhancement of the excretion of NO₂ + NO₃ was observed with the administration of TJ-114. Pre-intra-peritoneal injection of L-NAME inhibited the increase in urine volume, urinary NO₂ + NO₃, and urinary urea nitrogen in the rats treated with TJ-114. In contrast, the level of urine volume in the treatment of furosemide was not influenced by the pre-treatment with L-NAME

(Fig. 3). Furosemide tended to increase urinary NO₂ + NO₃ excretion, but its effect was not significant in any group.

MABP (Table 5)

MABP was unchanged by the treatment with TJ-114 and furosemide. However, MABP was significantly increased by the administration of L-NAME with or without TJ-114 and furosemide.

Discussion

In the present study, it was clarified that *Sairei-to* (TJ-114) increased urine volume in pentobarbital-

Fig. 2. Influence of L-NAME on diuretic effect of TJ-114 in pentobarbital-anesthetized rats. L-NAME (6 mg/kg) or saline was injected i.p. 5 min before the administration of TJ-114 (1.5 g/kg) or vehicle (distilled water). The levels of urinary NO₂ + NO₃ and urinary urea nitrogen for 60 min after TJ-114 administration are shown as a ratio (%) to the basal value before the treatment with L-NAME or saline. * $P < 0.05$, ** $P < 0.01$, significant difference compared with each group ($n = 6$) (Bonferroni test).

Fig. 3. Influence of L-NAME on diuretic effect of furosemide in pentobarbital-anesthetized rats. L-NAME (6 mg/kg) was injected i.p. 5 min before the administration of furosemide (FUR, 12 mg/kg). The levels of urinary NO₂ + NO₃ for 60 min after furosemide administration are shown as a ratio (%) to the basal value before the treatment with or without L-NAME. * $P < 0.05$, ** $P < 0.01$, significant difference compared with each group ($n = 4 - 6$) (Bonferroni test).

anesthetized rats. The change in urinary sodium excretion on treatment with TJ-114 was insignificant and slight as compared with the furosemide-treated group. However, the highest dose of TJ-114 significantly increased urinary urea nitrogen excretion; hence, it was considered that the change in urinary osmolality was minimal compared with that after furosemide admin-

istration.

Thus, in order to clarify the effect of TJ-114 on free water excretion, Cosm and $\text{Tc}_{\text{H}_2\text{O}}$ were calculated. $\text{Tc}_{\text{H}_2\text{O}}$ increased over twofold compared to the basal value with the increase in Cosm at the highest dose of TJ-114. However, recent reports (7–9) have demonstrated that electrolyte-free water clearance ($\text{E-C}_{\text{H}_2\text{O}}$) or $\text{E-Tc}_{\text{H}_2\text{O}}$

Table 5. Effects of TJ-114, furosemide, and L-NAME on mean arterial blood pressure in pentobarbital-anesthetized rats

		Mean arterial blood pressure (mmHg)		
Time after treatment		Basal	0 – 30 min	30 – 60 min
Exp. 1	Vehicle (distilled water)	141.4 ± 4.2	145.0 ± 4.7	141.4 ± 5.8
	TJ-114, 0.5 g/kg	140.3 ± 4.5	148.1 ± 4.7	143.6 ± 3.7
	TJ-114, 1.0 g/kg	140.3 ± 3.9	141.7 ± 3.1	142.5 ± 4.7
	TJ-114, 1.5 g/kg	134.8 ± 4.1	139.5 ± 3.8	139.0 ± 2.5
Exp. 2	Vehicle (distilled water)	136.9 ± 3.3	138.3 ± 3.2	140.0 ± 1.8
	L-NAME	130.8 ± 5.1	151.4 ± 7.5	156.4 ± 8.4 ^a
	TJ-114 (1.5 g/kg)	139.2 ± 4.5	141.1 ± 5.2	143.9 ± 6.9
	L-NAME + TJ-114	134.4 ± 2.6	164.2 ± 3.7 ^{b,c}	173.6 ± 3.6 ^{b,c}
Exp. 3	Vehicle (0.5% tween-80)	134.7 ± 1.3	135.0 ± 2.7	133.6 ± 1.5
	Furosemide	134.2 ± 4.3	138.1 ± 3.0	138.6 ± 4.0
	L-NAME + Furosemide	133.3 ± 1.7	146.1 ± 3.1	158.3 ± 4.4 ^{b,c}

Each value is expressed as a mean ± S.E.M. ^a*P*<0.05, ^b*P*<0.01, compared to the basal value; ^c*P*<0.01, compared to Vehicle with one-way ANOVA followed by Dunnett's test.

reflected the tonicity balance better than classic C_{H_2O} or Tc_{H_2O} in the treatment of electrolyte-abnormal patients. $E-C_{H_2O}$ is defined as the osmolar clearance of only effective osmoles (sodium, potassium, and their accompanying anions), not permeable osmoles that do not alter tonicity (urea, etc.). Since TJ-114 increased urinary urea nitrogen excretion, it seems that $E-Tc_{H_2O}$ is a more suitable index than classic Tc_{H_2O} to exclude the influence of ineffective osmoles on the measurement of free water excretion. $E-C_{osm}$ was significantly increased; however, $E-Tc_{H_2O}$ was not influenced by treatment with TJ-114. These findings suggest that the changes of urinary urea nitrogen concentration markedly influenced the value of Tc_{H_2O} , and it is possible that TJ-114 does not change free water excretion.

With regard to the mechanism underlying the diuretic effect of TJ-114, we examined whether it is related to NO. NO regulates the systemic and renal circulation as a potent vasodilator produced by the vascular endothelium. Moreover, it has been demonstrated that NO directly inhibited the sodium transporter in several tubule segments of nephrons in experiments *in vitro* (10, 11). NO also plays an important role in physiological processes such as excretory function in the kidney. In fact, it was shown that TJ-114 enhanced the urinary excretion of NO metabolites ($NO_2 + NO_3$) in the present study. It has been reported that the urinary $NO_2 + NO_3$ excretion was correlated to water/sodium excretion and increased following the application of most types of diuretics, suggesting that urinary levels of $NO_2 + NO_3$ do not correctly reflect intrarenal NO production (12). However, the urinary level of $NO_2 + NO_3$ in TJ-114-treated rats was higher than that in furosemide-treated

rats, in which the same change of urine volume was observed. Hence TJ-114 may activate endogenous NO production. Furthermore, in subsequent experiments, intraperitoneal pre-treatment with L-NAME, a NOS inhibitor, inhibited the increase in urine volume and urinary $NO_2 + NO_3$ in rats administered TJ-114. These same observations were made following intravenous injection of L-NAME. Any influence on the absorption and distribution of drugs from the duodenum by L-NAME can be excluded because intraperitoneal administration of L-NAME had no effect on the diuretic response to furosemide. Therefore, these findings suggest that the diuretic effect of TJ-114 is mediated by NO production. On the other hand, TJ-114 increased urinary urea nitrogen levels and the effect was inhibited by pre-intraperitoneal injection of L-NAME. It has been reported that the urea filtered from glomeruli was reabsorbed passively in renal proximal tubules during the reabsorption of water or sodium (13, 14). Thus, it is considered that the increase in urinary urea nitrogen with TJ-114 is associated with the secretion of water and sodium in proximal tubules.

In the present study, TJ-114 had an NO-related diuretic effect, but it did not change systemic blood pressure. Lahera et al. (15) observed that the effects of NO on renal excretory function were independent of systemic hemodynamic responses. We can speculate that the vasodilatory effect of TJ-114 disappears due to the counter-regulation of endogenous vasoconstrictor substances in pentobarbital-anesthetized rats. In addition, it has been demonstrated that NO modulated renal circulation (16, 17). The effect of TJ-114 on the renal circulation will need to be determined in future studies.

In conclusion, it was clarified that TJ-114 increased urine volume in pentobarbital-anesthetized rats. This effect was inhibited by pre-treatment with L-NAME; therefore, the stimulation of NO production may contribute to the diuretic effect of TJ-114.

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