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Effects of Gorei-san: A Traditional Japanese Kampo Medicine, on Aquaporin 1, 2, 3, 4 and V2R mRNA Expression in Rat Kidney and Forebrain

T. Kurita, K. Nakamura, M. Tabuchi, M. Orita, K. Ooshima and H. Higashino

The details of pharmacological mechanisms of Gorei-san, a traditional Japanese Kampo medicine, remains to be clarified, although it has been used for diuretic and hydrostatic purposes. From these circumstances, the effects of this medicine on the expressions of aquaporin (AQP) 1, 2, 3, 4 and vasopressin 2 receptor (V2R) mRNAs were investigated in relation to diuresis and water balance regulation in the kidney and brain. Gorei-san extract decocted with hot water was given to rats loaded with 50 mL kg⁻¹ volume of physiological saline and AQP1, 2, 3, 4 and V2R mRNAs were measured with real-time polymerase chain reaction (PCR) in the cortex and the medulla of kidney and the forebrain. A low dose of Gorei-san extract (100 mg kg⁻¹) led to an increase in urine excretion and lower AQP3 mRNA expression in the cortex as well as lower expression of AQP2 and AQP3 mRNAs in the medulla of kidney, whereas no change in V2R mRNA expression was observed. AQP1 mRNA expression decreased in the forebrain of rats loaded with an excess volume of physiological saline compared with rats not loaded with excess saline and given no agent. A low dose of Gorei-san extract increased urine excretion volume, probably due to the downregulation of AQP3 mRNA in the cortex and downregulation of AQP2 and AQP3 mRNAs in the medulla of the kidney, in which changes were not related to V2R mRNA expression. An excess volume of physiological saline given to rats caused an inhibition of AQP1 mRNA expression in the forebrain, which probably functioned to maintain the water balance in a hyper-hydrous state.

Key words: Gorei-san, aquaporin, vasopressin 2 receptor, diuretic effect, hydrostatic action, kidney

INTRODUCTION

Thirteen aquaporins as water permeability molecules have been discovered in recent days in mammals and at least eight aquaporins (AQP1, 2, 3, 4, 6, 7, 8 and 11) in the kidney (Kwon *et al.*, 2009) and at least three (AQP1, 4 and 9) are expressed in the brain (Zelenina, 2010). Of these AQPs, four AQPs (AQP1, 2, 3 and 4) are distributed in different regions of the kidney and play important roles in regulating water balance in the body (Kwon *et al.*, 2009). AQP1, which is expressed in the proximal tubules and descending thin limbs, plays an important role in the water channels that function in the water reabsorption process (Preston and Agre, 1991; Agre *et al.*, 1993; Nielsen *et al.*, 1993). It has been reported that AQP2, 3 and 4 are expressed in principle cells of the collecting duct in the kidney. And, the expression of AQP2 on the plasma membrane and AQP3 on the basolateral membrane of the principle cells is regulated by vasopressin stimuli during body water balance (Fushimi *et al.*, 1993; Echevarria *et al.*, 1994).

Gorei-san, a well-known traditional Japanese medicine formula, has been used as a hydrostatic modulating drug to treat the symptoms of urine volume decreases including edema, thirst, spontaneous sweating and oliguria (Sato *et al.*, 2005). Gorei-san is a mixture of five different medicinal plants: *Alisma plantago-aquatica* var. *orientale* rhizoma, *Atractylodes lancea* rhizoma, *Poria cocos* WOLF sclerotia, *Polyporus umbellatus* FRIES sclerotia and *Cinnamomum cassia* bark. Gorei-san is believed to have two medicinal actions: one is a diuretic action (Yoshizawa *et al.*, 2003; He *et al.*, 2008; Miyagami and Kagawa, 2009) and the other is a hydrostatic modulating effect in which movement of water molecules are adjusted (Ohnishi *et al.*, 2000). In these recent studies, AQP molecules have been discussed in relation to the hydrostatic modulating mechanism caused by Gorei-san (Isohama, 2006; Zhang *et al.*, 2010).

The antidiuretic hormone vasopressin regulates water permeability by binding to V2R on the surface of cells in the distal convoluted tubules and collecting structures of the kidney. This action occurs through trafficking of phosphorylated AQP2 to the apical plasma membrane (Kwon *et al.*, 2009). However, the details of the pharmacological mechanisms of Gorei-san remain to be clarified. Therefore, the current study examined the effects of Gorei-san on the expression of four types of AQP and V2R mRNAs in the kidney and the brain, where hydration and dehydration effects are particularly important. We compared Gorei-san with two representative diuretics: furosemide, a loop diuretic and acetazolamide, a carbonic anhydrase inhibitor.

MATERIALS AND METHODS

This study was conducted in a period of September 1, 2007 to September 30, 2010 for 3 years at place of the Department of Pharmacology, Kinki University School of Medicine, Osaka, Japan.

Animals: Male 6-week-old Sprague-Dawley thirty six rats weighing 170-200 g were purchased from CLEA Japan Inc. (Tokyo, Japan) and kept under lighting control (light and dark phases for 12 h each) at a temperature of $22\pm 2^{\circ}\text{C}$ and in 60% humidity. Rats were used for experiments after 2 weeks of acclimatization. All experiments were conducted in accordance with the Guiding Principles for Care and Use of Animals of the Physiological Society of Japan and were approved by the Institutional Animal Use and Care Committee of Kinki University School of Medicine (KAME-19-078).

Materials: Dry powder of Gorei-san extract decocted with hot water was provided by Tsumura Co. (Tokyo, Japan), a representative pharmaceutical company of traditional Japanese Kampo medicine. In accordance with the company's instructions, 2.0 g of dried powder was extracted with hot water from a mixture of the following five medicinal plants: 4.0 g of *Alisma plantago-aquatica* var. *orientale* rhizoma, 3.0 g of *Atractylodes lancea* rhizoma, 3.0 g of *Poria cocos* WOLF sclerotia, 3.0 g of *Polyporus umbellatus* FRIES sclerotia and 1.5 g of *Cinnamomum cassia* bark. Furosemide was purchased from Sigma Co., Ltd. (St. Louis, MO, USA) and acetazolamide was purchased from Sanwa Kagaku Kenkyusyo Co., Ltd. (Tokyo, Japan). Each compound was dissolved in physiological saline at concentrations of 10 mg mL^{-1} for low-dose Gorei-san, 30 mg mL^{-1} for high-dose Gorei-san, 1 mg mL^{-1} for furosemide and 5 mg mL^{-1} for acetazolamide.

Grouping and procedures for administration of compounds: Rats were randomly divided into the following six groups of six rats each: control A group (Cont A) was loaded with physiological saline without test compound; control B group (Cont B) was not loaded with physiological saline or test compound; low-dose Gorei-san ($100\text{ mg kg}^{-1}\text{ BW}$) group (Low-G) with loading of physiological saline; high-dose Gorei-san ($300\text{ mg kg}^{-1}\text{ BW}$) group (High-G) with loading of physiological saline; furosemide ($10\text{ mg kg}^{-1}\text{ BW}$) group (Furosem) with loading of physiological saline; and acetazolamide ($50\text{ mg kg}^{-1}\text{ BW}$) group (Acetazm) with loading of physiological saline. Each animal was housed in an individual metabolic cage and was allowed free

access to water and standard rat chow (CE-2, Clea Japan Co., Tokyo, Japan) for 24 h. Animals fasted overnight with free access to water before treatments.

On the 1st day, the Cont A, Low-G, High-G, Furosem and Acetazm groups were given 2 mL 100 g⁻¹ BW of physiological saline orally with a gastric tube two times at intervals of 5 min. And then, compounds (100 mg kg⁻¹ BW Gorei-san, 300 mg kg⁻¹ BW Gorei-san, 10 mg kg⁻¹ BW furosemide and 50 mg kg⁻¹ BW acetazolamide) were given orally to the Low-G, High-G, Furosem and Acetazm groups, respectively. Rats in the Cont A group were given 1 mL 100 g⁻¹ BW physiological saline without any compound. After these treatments, excreted urine was collected in a metabolic cage housing a single rat.

On the 2nd and 3rd days, the same treatments were repeated as on the 1st day. Four hours after the last treatment on the 3rd day, rats were anesthetized with an intra-peritoneal injection of 50 mg kg⁻¹ sodium pentobarbital and the abdominal skin was incised with scissors. The kidneys and brains were washed with 10 mL of ice-cold sterile ribonuclease (RNase)-free physiological saline through the descending aorta to remove blood cells and the organs were quickly removed from the body.

RNA isolation, cDNA synthesis and real-time PCR:

Tissues were immediately submerged in RNA Later (QIAGEN, Hilden, Germany) and incubated at 4°C overnight and stored at -40°C until used. After homogenization with a Tissue Lizer (QIAGEN), total RNA was isolated with an RNeasy Mini kit (QIAGEN) according to the manufacturer's instructions. First-strand cDNA was synthesized with a Prime Script RT Master Mix kit (TaKaRa, Shiga, Japan). Real-time PCR was performed with a SYBR Premix Ex Taq II kit (TaKaRa) and each appropriate primer as described below and the amounts of synthesized DNA fragments were measured with an ABI Prism 7900 HT Sequence Detection System (ABI, Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows. An initial denaturation was performed at 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 30 sec. Primers used were as follows:

AQP1-F: ATTGGGTCAAGCTCAGCAGTCA
AQP1-R: TTAGCCATTGTCCAGGCAGAAAC
AQP2-F: GACCATGCAGCTGGTGCTGT
AQP2-R: TCATGGAGCAACCGGTGAAAT
AQP3-F: CACAGCTGGTATCTTTGCCACCTA
AQP3-R: AATAAGGGCTGCTGTGCCTATGA
AQP4-F: AGGCAATGTGTGCACTGCTCTAAC
AQP4-R: GAAGGTGTCAACGTCACACAACAA
V2R-F: CCGTGAGGATGACACTGGTGA
V2R-R: TCCAAGGGTTGGTACAGCTGTAAAG

β-actin-F: GGAGATTACTGCCCTGGCTCCTA
β-actin-R: GACTCATCGTACTCCTGCTTGCTG
GAPDH-F: GGCACAGTCAAGGCTGAGAATG
GAPDH-R: ATGGTGGTGAAGACGCCAGTA

mRNA expression levels of tested genes were normalized to levels of GAPDHmRNA in the kidney and β-actin mRNA in the forebrain because of the differences in abundant expression in each tissue.

Statistical analysis: Data were expressed as the Mean± SEM. Significant differences between two groups were evaluated with a Student's t-test. Multiple comparisons among several groups were made with one-way analysis of variance (ANOVA) followed by a Dunnett's test (Dunnett and Tamhane, 1992). A probability value of p<0.05 was considered as statistically significant.

RESULTS

The effects of Gorei-san extract, furosemide and acetazolamide on the urinary excretion volumes at 2, 4, 6 and 24 h after compound administration in the rats are shown in Fig. 1. The volume of urine increased with time in every group. Although low-dose Gorei-san extract (100 mg kg⁻¹) was not different compared to the untreated control A group at 2, 4 and 6 h, a significant increase was observed at 24 h (p<0.05). On the other hand, treatment with high-dose (300 mg kg⁻¹) Gorei-san extract had no significant effect on urine excretion. Furosemide (10 mg kg⁻¹) increased the urine volume at 4 and 6 h (p<0.05) and acetazolamide (50 mg kg⁻¹) produced diuresis at all four time points (p<0.01) compared with the control A group.

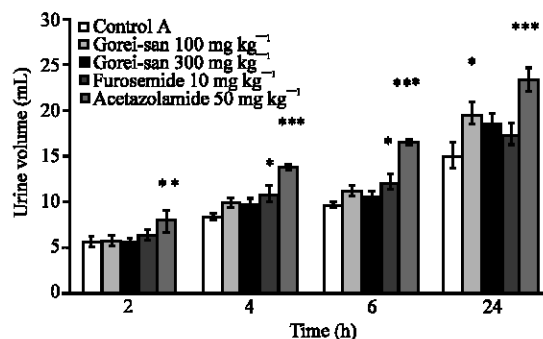


Fig. 1: Effects of the various compounds on the excreted urine volumes at 2, 4, 6 and 24 h after administration. Values are expressed as the Mean±SEM (n = 6). *p<0.05, **p<0.01 and ***p<0.001: vs. control A group, analyzed by ANOVA with Dunnett's test

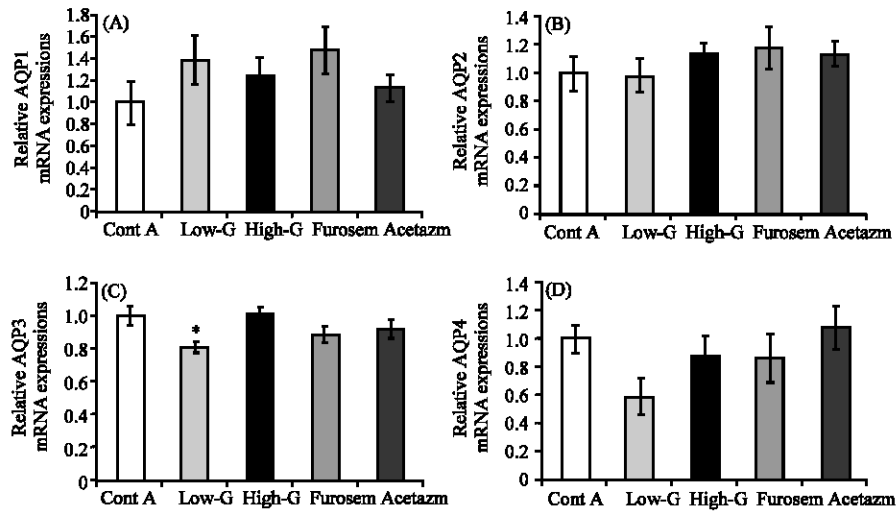


Fig. 2: Relative mRNA expression of AQP1, AQP2, AQP3 and AQP4 in the cortex of the kidney following administration of no compound (control A: Cont A), 100 mg kg⁻¹ Gorei-san (Low-G), 300 mg kg⁻¹ Gorei-san (High-G), 10 mg kg⁻¹ furosemide (Furosem), or 50 mg kg⁻¹ acetazolamide (Acetazm) with physiological saline loading (50 mL kg⁻¹). The expression level of each mRNA was normalized to GAPDH mRNA expression. Values are expressed as the Mean±SE (n = 6) *p<0.05: vs. control A group, analyzed by ANOVA with Dunnett's test

AQP1, 2, 3 and 4 mRNA expressions in the cortex of kidney: To access the effects of Gorei-san extract, furosemide and acetazolamide on renal gene expression associated with water regulation, we quantitatively measured gene expression in the kidney and the forebrain with real-time PCR for genes involved in water permeability and re-absorption through AQP1, AQP2, AQP3 and AQP4 water channels and V2R.

The effects of Gorei-san extract, furosemide and acetazolamide on the relative gene expression in the cortex of the kidney are shown in Fig. 2 (A: AQP1 mRNA, B: AQP2 mRNA, C: AQP3 mRNA and D: AQP4 mRNA). In the cortex of the kidney, the relative mRNA expression of AQP1, AQP2 and AQP4 was not affected by the administration of the three compounds (Fig. 2A, B, D). On the other hand, the low-dose Gorei-san extract group showed a significantly lower AQP3 mRNA expression compared with that of the control A group (p<0.05). AQP3 mRNA expression was not different in the other groups (Fig. 2C).

AQP1, 2, 3 and 4 mRNA expressions in the medulla of kidney: In the medulla of the kidney, the relative AQP1 and AQP4 mRNA expression levels were not affected by the administration of the three compounds (Fig. 3A, D). On the other hand, the low-dose Gorei-san extract group showed significantly lower expression of AQP2 and AQP3 mRNAs compared with those of the control A group (p<0.05). AQP2 and AQP3 mRNA expressions were not different in the other groups (Fig. 3B, C).

V2R mRNA expression in the kidney: V2R mRNA expression in the cortex and the medulla of the kidney was not affected by any compound tested including low and high doses of Gorei-san extract, furosemide and acetazolamide (Fig. 4A, B).

AQP1, 2, 3, 4 and V2R mRNA expressions in the forebrain: We measured AQP1, AQP2, AQP3, AQP4 and V2R mRNA expressions in the forebrain of rats given Gorei-san extract, furosemide and acetazolamide. AQP2, AQP3 and V2R mRNA expression levels were too low to detect in contrast to the kidney. Although relative AQP1 and AQP4 mRNA expressions were detected in the forebrain, there were no significant differences between the control A group and each group given a compound.

Comparison between control A and B groups: Rats in the control A group that were given no compound and the other groups that were given the various compounds were orally loaded with excess physiological saline (50 mL kg⁻¹ BW) to induce diuresis and examine the effects of Kampo medicine in a hyper-hydrous state. Excess physiological saline may affect the mRNA expression of AQPs and V2R in addition to the effects of the compounds. Therefore, the differences between the effects of excess physiological saline and no saline were examined. The urine volumes in the excess physiological saline loading group (control A) and the no saline group (control B) are shown in Fig. 5. The control A group showed significantly more urine volume at 2, 4, 6 and

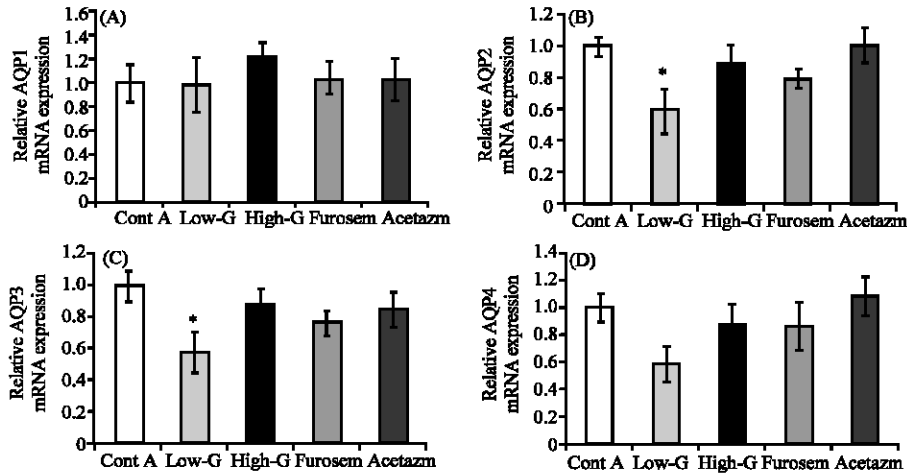


Fig. 3: Relative mRNA expression of AQP1, AQP2, AQP3 and AQP4 in the medulla of the kidney following administration of no compound (control A: Cont A), 100 mg kg⁻¹ Gorei-san (Low-G), 300 mg kg⁻¹ Gorei-san (High-G), 10 mg kg⁻¹ furosemide (Furosem), or 50 mg kg⁻¹ acetazolamide (Acetazm) with physiological saline loading (50 mL kg⁻¹). The expression level of each mRNA was normalized to GAPDH mRNA expression. Values are expressed as the Mean±SEM (n = 6). *p<0.05: vs. control A group, analyzed by ANOVA with Dunnett's test

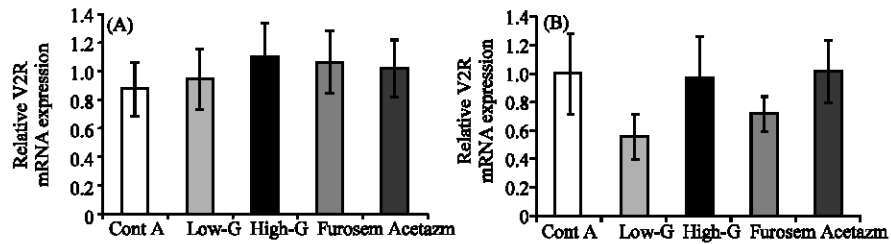


Fig. 4: Relative V2R mRNA expression in the cortex (A) and medulla (B) of the kidney following administration of no compound (control A: Cont A), 100 mg kg⁻¹ Gorei-san (Low-G), 300 mg kg⁻¹ Gorei-san (High-G), 10 mg kg⁻¹ furosemide (Furosem), or 50 mg kg⁻¹ acetazolamide (Acetazm) with physiological saline loading (50 mL kg⁻¹). The expression level of each mRNA was normalized to GAPDH mRNA expression. Values are expressed as the Mean±SEM (n = 6)

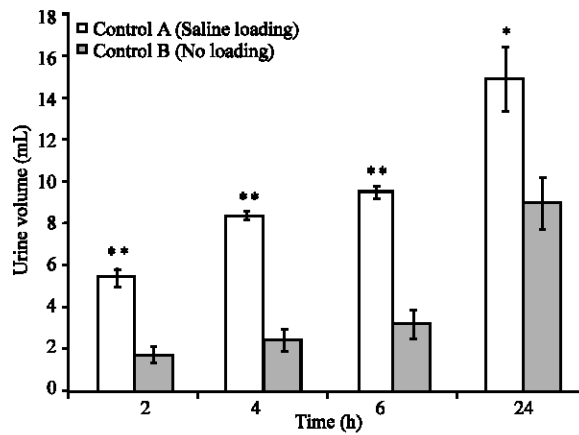


Fig. 5: Effects of excess loading of physiological saline (50 mL kg⁻¹ BW) on urine excretion volumes. Values are expressed as the Mean±SEM (n = 6) *p<0.05 and **p<0.001: vs. Control B, analyzed by Student's t-test

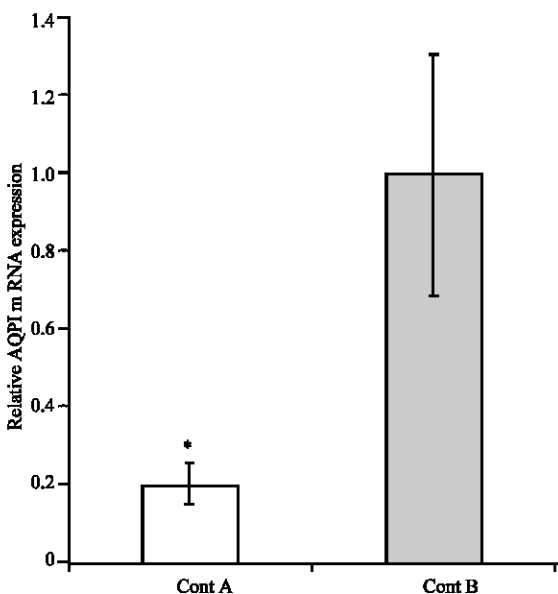


Fig. 6: Comparison of relative AQP1 mRNA expression in brain in the Control A group (loaded with 50 mL kg⁻¹ of physiological saline) and the Control B group (not loaded). The expression level of each mRNA was normalized to β -actin mRNA expression. Values are expressed as the Mean \pm SEM (n = 6) *p<0.05: vs. control B group, analyzed by Student's t-test

24 h compared with the control B group without saline. When AQP1, 2, 3, 4 and V2R mRNA expressions were compared between these two control groups, no difference was observed in the cortex or the medulla of the kidney. On the other hand, in the forebrain, AQP1 mRNA expression decreased in the control A group compared that in the control B group (p<0.05; Fig. 6), but the levels of AQP2, 3, 4 and V2R mRNA expression were not different between the two control groups.

DISCUSSION

In this study, we examined the effects of Gorei-san, a traditional Japanese Kampo medicine used for diuretic or hydrostatic purposes and compared the effects to two representative diuretics, furosemide (a loop diuretic) and acetazolamide (a carbonic anhydrase inhibitor). We looked at the levels of AQP and V2R mRNA and observed the following. (1) Low-dose Gorei-san extract (100 mg kg⁻¹) led to increased urine excretion 24 h after administration, whereas high-dose (300 mg kg⁻¹) Gorei-san extract did not. (2) Doses of 10 mg kg⁻¹ furosemide and 50 mg kg⁻¹ acetazolamide produced diuresis earlier and more than in the control A group. (3)

Low-dose Gorei-san extract resulted in lower AQP3 mRNA expression in the cortex of the kidney compared with that of the control A group, but this effect was not observed in other groups. (4) Low-dose Gorei-san extract led to lower expression of AQP2 and AQP3 mRNAs in the medulla of the kidney compared with expression in the control A group, but this effect was not observed in other groups. (5) No compound changed the expression of V2R mRNA in the cortex or the medulla of the kidney. (6) AQP2, 3 and V2R mRNA expression levels were too low to be detected in the forebrain. AQP1 and AQP4 mRNA expressions were detectable in the forebrain, but no difference was observed between the control A group and the groups given compounds. (7) AQP1 mRNA expression decreased in the forebrain of the control A group, which was loaded with excess physiological saline, compared with the control B group, which received no excess saline; however, AQP1, 2, 3, 4 and V2R mRNA expression did not change in the cortex or medulla of the kidney under these conditions.

Gorei-san, a well-known traditional Japanese Kampo medicine formula, has been used for inducing hydrostatic modulating and diuretic effects (Sato *et al.*, 2005). Gorei-san has been shown to have diuretic effects in rats (Haranaka *et al.*, 1981), mice (Ohnishi *et al.*, 2000; Tanaka *et al.*, 1984) and it also has an anti-diuretic effect in dehydrated conditions (Ohnishi *et al.*, 2000). There is a report not to have those effects in KKAY mice given Gorei-san (Morimoto *et al.*, 2002). Although many researchers reported that Gorei-san have a diuretic effects, opposite results were also shown in some conditions as mentioned above.

In recent studies, AQP molecules related to the hydrostatic modulating mechanism have been shown to be induced by Gorei-san. Thirteen types of AQPs have been identified. At least eight AQPs (AQP1, 2, 3, 4, 6, 7, 8 and 11) are expressed in the mammalian kidney (Kwon *et al.*, 2009) and at least three (AQP1, 4 and 9) are expressed in the brain (Zelenina, 2010). Of these AQPs, four AQPs (AQP1, 2, 3 and 4) are distributed in different regions of the kidney and play important roles in regulating water balance in the body (Kwon *et al.*, 2009). AQP1, which is expressed in the proximal tubules and descending thin limbs, plays an important role in the water channels that function in the water reabsorption process (Preston and Agre, 1991; Agre *et al.*, 1993; Nielsen *et al.*, 1993). It has been reported that AQP2, 3 and 4 are expressed in principle cells of the collecting duct in the kidney. And, the expression of AQP2 on the plasma membrane and AQP3 on the basolateral membrane of the principle cells is regulated by vasopressin stimuli during body water balance (Fushimi *et al.*, 1993; Echevarria *et al.*,

1994). Vasopressin regulates water permeability by binding to V2R on the collecting tubules. Two time course mechanisms are thought to play a role in regulating water permeability. The short-term mechanism is regulated by trafficking of AQP2 from intracellular vesicles to the apical membrane of principle cells (Nielsen *et al.*, 1995), whereas the long-term mechanism is regulated by AQP2 and AQP3 synthesis in principle cells (DiGiovanni *et al.*, 1994; Ecelbarger *et al.*, 1995). In our study, we could not detect higher expression of V2R mRNA upon Gorei-san administration in the kidney. Therefore, Gorei-san will downregulate the expressions of AQP2 and/or AQP3 mRNAs directly in the kidney, or induces arginine vasopressin secretion, not be measured here, from the neurohypophysis to cause diuretic action. AQP4 is present on the basolateral membrane of the kidney (Terris *et al.*, 1995) and is strongly expressed in astrocyte endfeet in the brain, where it may play an important role in brain edema formation (Nielsen *et al.*, 1997).

Isohama (2006) reported that Gorei-san inhibits water permeability in MLE-12 cells by inhibiting AQP molecules in vitro and that Mn^{2+} and Zn^{2+} ions are involved in this inhibition process that is mediated by AQPs. In the brain edema model, Gorei-san increases the survival time of rats by inhibiting water permeability that is mediated by AQP4. Zhang *et al.* (2010) showed that *Polyporus umbellatus* FRIES sclerotia, which is a component of the Gorei-san formula, causes downregulation of AQP2 production by downregulating V2R. Nevertheless, the details of the pharmacological mechanism of Gorei-san remain unclear.

Low-dose Gorei-san extract downregulated the expression of AQP3 mRNA in the cortex and AQP2 and AQP3 mRNAs in the medulla of the kidney. Synthesis of AQP2 and AQP3 mRNAs that is associated with vasopressin occurs in the collecting duct of the kidney (DiGiovanni *et al.*, 1994; Ecelbarger *et al.*, 1995). However, present findings suggest that AQP3 is involved in the diuretic process together with AQP2 in either the cortex or the medulla of the kidney and is not related to V2R because V2R mRNA expression was not changed in our experiment.

Furosemide and acetazolamide produced apparent diuresis, but expression of AQPs and V2R mRNAs was not affected by these drugs. Two representative diuretics with different mechanisms of action, such as furosemide and acetazolamide, did not show any apparent effects on expression of the four types of AQP and V2R mRNAs in the kidney or forebrain in our study which is in contrast to previous reports regarding furosemide. Furosemide (10 mg kg^{-1}) downregulates AQP2 mRNA expression after 8 days of administration (Zhang *et al.*, 2010). However, another group reported that this drug (20 mg kg^{-1})

upregulates AQP2 in the kidney of rats after 8 days of administration (Lin *et al.*, 2007). In our experiments, the two diuretics, furosemide and acetazolamide, caused diuresis without affecting AQP expression.

Although low-dose Gorei-san extract (100 mg kg^{-1}) caused diuretic effects and led to downregulation of AQP2 and AQP3 mRNAs with an excess volume of physiological saline (50 mL kg^{-1}), high-dose Gorei-san extract (300 mg kg^{-1}) did not induce this effect. The reason may be that the absorption of the effective compounds with the higher dose of Gorei-san extract from the intestinal membranes may be delayed because 2-3 mL of 30 mg mL^{-1} Gorei-san extract dissolved in physiological saline raise has an osmotic pressure by 30-50 mEq L, which is higher than that of physiological saline. Thus, the diuretic effect of Gorei-san may not be dominant compared to its usual function. Of note, diuresis was observed in preliminary experiments with large 19-week-old SD rats weighing 400-500 g which absorbed more fluid volume than small rats, because of the less loading in larger rats than small rats.

In the brain, AQP 2, 3 and V2R mRNAs were not detectable. Thus, these two types of AQPs and V2R may not be important for maintaining brain water balance functions. Although Gorei-san extract did not affect the expression of the four types of AQP and V2R mRNAs in the brain, excess loading of physiological saline into the rats caused an inhibition of AQP1 mRNA expression. Several reports have examined AQP1, osmotic pressure and water transport in the brain. AQP1 is selectively expressed on the apical surface of the choroid plexus epithelium (Speake *et al.*, 2003). Osmotically induced water transport is rapid in the choroid plexus of wild-type mice and water transport is reduced by five-fold in AQP1 null mice (Oshio *et al.*, 2004). Intracranial pressure in a model of focal brain injury in AQP1 null mice is remarkably reduced and survival rates are higher compared with wild-type mice. In systemic hyponatremia, AQP1 expression is significantly increased after 2 h and then attenuated after 6 h (Moon *et al.*, 2006). These results indicate that AQP1 molecules maintain water homeostasis in the brain under hypotonic and hypertonic stresses by regulating cerebrospinal fluid (CSF) dynamics. Therefore, AQP1 molecules also likely regulate water transport in the forebrain, as we examined here, as well as in the choroid plexus epithelium. Welch (1963) discovered that acetazolamide decreases the relative rate of CSF secretion. The trend towards lower expression of AQP1 mRNA, but not a significant change, in the forebrain of the acetazolamide group in our study suggests a preventive effect on water imbalance. Relative expression levels of mRNA in the cortex vs. the medulla of kidney

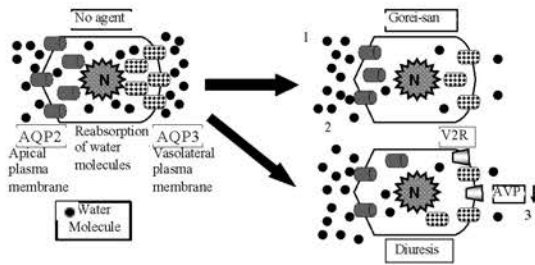


Fig. 7: The summary of diuretic action of Gorei-san in principal cells of the collecting duct in the kidney

were 0.900 ± 0.100 for AQP1, 0.052 ± 0.004 for AQP2, 0.092 ± 0.007 for AQP3, 0.021 ± 0.003 for AQP4 and 0.091 ± 0.004 for V2R. Thus, expression of AQP2, 3, 4 and V2R mRNA was significantly lower in the cortex compared with the medulla, suggesting that AQP2, 3, 4 and V2R have more important functions in the medulla than in the cortex of the kidney.

CONCLUSION

Low-dose Gorei-san extract (100 mg kg^{-1}) increased urine volume, probably due to the downregulation of AQP3 mRNA in the cortex and downregulation of AQP2 and AQP3 mRNAs in the medulla of the kidney as shown in Fig. 7. V2R did not appear to be related to these reductions of AQPs in the kidney. AQP 2, 3 and V2R mRNAs were not detected in the brain, indicating the low importance of these proteins for maintaining brain functions. An excess volume of physiological saline caused an inhibition of AQP1 mRNA expression in the brain, probably to maintain water homeostasis under hyper-hydrous stress.

REFERENCES

Agre, P., G.M. Preston, B.L. Smith, J.S. Jung and S. Raina *et al.*, 1993. Aquaporin CHIP: The archetypal molecular water channel. *Am. J. Physiol.*, 265: F463-F476.

DiGiovanni, S.R., S. Nielsen, E.I. Christensen and M.A. Knepper, 1994. Regulation of collecting duct water channel expression by vasopressin in Brattleboro rat. *Proc. Natl. Acad. Sci. USA.*, 91: 8984-8988.

Dunnett, C.W. and A.C. Tamhane, 1992. Comparisons between a new drug and active and placebo controls in an efficacy clinical trial. *Stat. Med.*, 11: 1057-1063.

Ecelbarger, C.A., J. Terris, G. Frindt, M. Echevarria, D. Marples, S. Nielsen and M.A. Knepper, 1995. Aquaporin-3 water channel localization and regulation in rat kidney. *Am. J. Physiol.*, 269: F663-F672.

Echevarria, M., E.E. Windhager, S.S. Tate and G. Frindt, 1994. Cloning and expression of AQP3, a water channel from the medullary collecting duct of rat kidney. *Proc. Natl. Acad. Sci. USA.*, 91: 10997-11001.

Fushimi, K., S. Uchida, Y. Harat, Y. Hirata, F. Marumo and S. Sasaki, 1993. Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature*, 361: 549-552.

Haranaka, R., S. Watabe, R. Kohashi, K. Hiraide and J. Maruta *et al.*, 1981. The effect of the Chinese herb diuretics (Goreisan, Choreito, Saireito) in growing rats: Part I. *Proc. Symp. WAKAN-YAKU*, 14: 105-110.

He, L., X. Rong, J.M. Jiang, P.Q. Liu and Y. Li, 2008. Amelioration of anti-cancer agent adriamycin-induced nephrotic syndrome in rats by Wulingsan (Gorei-San), a blended traditional Chinese herbal medicine. *Food Chem. Toxicol.*, 46: 1452-1460.

Isohama, Y., 2006. Aquaporin Modification: A new molecular mechanism to concern *J. Pharm. Soc. Jpn.*, 126: 70-73.

Kwon, T.H., J. Nielsen, H.B. Moller, R.A. Fenton, S. Nielsen and J. Frokiaer, 2009. Aquaporin in the kidney. *Handb. Exp. Pharmacol.*, 190: 95-132.

Lin, S., D.L. Xu, W.Y. Lai, Q.B. Shen, J.X. Gao, B. Zhang and H. Ren, 2007. Effects of furosemide, antisterone and hydrochlorothiazide on expression of kidney aquaporin-2 gene and urine aquaporin-2 excretion in rats. *Nan Fang Yi Ke Da Xue Xue Bao*, 27: 802-804.

Miyagami, M. and Y. Kagawa, 2009. Effectiveness of kampo medicine gorei-san for chronic subdural hematoma. *No Shinkei Geka*, 37: 765-770.

Moon, Y., S.J. Hong, D. Shin and Y. Jung, 2006. Increased aquaporin-1 expression in choroid plexus epithelium after systemic hyponatremia. *Neurosci. Lett.*, 395: 1-6.

Morimoto, Y., M. Sakata, A. Ohno, T. Maegawa and S. Tajima, 2002. Effects of Byakko-ka-ninjin-to, Bofu-tsusho-san and Gorei-san on blood glucose level, water intake and urine volume in KKAY mice. *Yakugaku Zasshi*, 122: 163-168.

Nielsen, S., B.L. Smith, E.I. Christensen, M.A. Knepper and P. Agre, 1993. CHIP28 water channels are localized in constitutively water-permeable segments of the nephron. *J. Cell Biol.*, 120: 371-383.

Nielsen, S., C.L. Chou, D. Marples, E.I. Christensen, B.K. Kishore and M.A. Knepper, 1995. Vasopressin increases water permeability of kidney collecting duct by inducing *Proc. Natl. Acad. Sci. USA.*, 92: 1013-1017.

- Nielsen, S., E.A. Nagelhus, M. Amiry-Moghaddam, C. Bourque, P. Agre and O.P. Ottersen, 1997. Specialized membrane domains for water transport in glial cells: High-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J. Neurosci.*, 17: 171-180.
- Ohnishi, N., K. Nagasawa and T. Yokoyama, 2000. The verification of regulatory effects of Kampo formulations on body fluid using model mice. *J. Trad. Med.*, 17: 131-136.
- Oshio, K., H. Watanabe, Y. Song, A.S. Verkman and G.T. Manley, 2004. Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel Aquaporin-1. *FASEB J.*, 19: 76-78.
- Preston, G.M. and P. Agre, 1991. Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: Member of an ancient channel family. *Proc. Natl. Acad. Sci. USA.*, 88: 11110-11114.
- Sato, Y., T. Hanawa, M. Arai, J. Cyong and M. Fukuzawa *et al.*, 2005. Introduction to KAMPO. Japanese Medicine. Elsevier Japan K.K., Tokyo, pp: 134.
- Speake, T., L.J. Freeman and P.D. Brown, 2003. Expression of aquaporin 1 and aquaporin 4 water channels in rat choroid plexus. *Biochim. Biophys. Acta*, 1609: 80-86.
- Tanaka, S., T. Akira and M. Tabata, 1984. Pharmacological analysis of the traditional Chinese prescription Goreisan-ryo. *Yakugaku Zasshi*, 104: 601-606.
- Terris, J., C.A. Ecelbarger, D. Marples, M.A. Knepper and S. Nielsen, 1995. Distribution of aquaporin-4 water channel expression within rat kidney. *Am. J. Physiol.*, 269: F775-F785.
- Welch, K., 1963. Secretion of cerebrospinal fluid by choroid plexus of the rabbit. *Am. J. Physiol.*, 205: 617-624.
- Yoshizawa, C., M. Mikage and A. Taru, 2003. A study of the medical treatment for Ho-shun-in, Mrs. Toshiie Maeda, described in *Igaku-tensho-ki*. *Yakushigaku Zasshi*, 38: 82-92.
- Zelenina, M., 2010. Regulation of brain aquaporins. *Neurochem. Int.*, 57: 468-488.
- Zhang, G., X. Zeng, L. Han, J.A. Wei and H. Huang, 2010. Diuretic activity and kidney medulla AQP1, AQP2, AQP3, V2R expression of the aqueous extract of sclerotia of *Polyporus umbellatus* FRIES in normal rats. *J. Ethnopharmacol.*, 128: 433-437.