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Review Article

Herbal medicine Oryeongsan (Wulingsan): Cardio-renal effects via modulation of renin-angiotensin system and atrial natriuretic peptide system

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ABSTRACT

Background: Oryeongsan (Wulingsan, Goreisan) has long been used for the treatment of impaired body fluid metabolism. However, the action mechanisms have not been clearly defined. Recently, effects of Oryeongsan on the body fluid and Na^+ metabolism and the action mechanisms have been shown more clearly. The present review focuses on the recent findings on the effects of Oryeongsan in the cardio-renal system in relation with body fluid metabolism and action mechanisms leading to a decrease in blood pressure in animal models of hypertension.
Methods: The new and recent findings were searched by using searching systems including PubMed-NCBI and Google-Scholar.
Results: Oryeongsan induced an increase in glomerular filtration rate, and natriuresis and diuresis with a decreased osmolality and resulted in a contraction of the body fluid and Na^+ balance. These findings were associated with a suppression of abundance of Na^+-H^+-exchanger isoform 3 expression and V_2 receptor/aquaporin2 water channel signaling pathway in the kidney. Further, treatment with Oryeongsan accentuated atrial natriuretic peptide secretion in the atria from spontaneously hypertensive rats in which the secretion was suppressed. In addition, Oryeongsan ameliorated impaired vasodilation in spontaneously hypertensive rats.
Conclusion: The effects of Oryeongsan in the kidney, atria, and vessel were accompanied by a suppression of AT_1 receptor and concurrent accentuation of abundance of AT_2/Mas receptors expression and modulation of the natriuretic peptide system in these organs from hypertensive rats. The review shows multiple sites of action of Oryeongsan and mechanisms involved in the regulation of volume and pressure homeostasis in the body.

1. Introduction

Herbal formula Oryeongsan (Wulingsan, Goreisan) has long been used for the treatment of imbalance of body fluid homeostasis including edema. The formula is composed of five medicinal herbs. Although the ratios in the amount of each component herb are slightly different among the different areas, the formulas, Oryeongsan (ORS, in Korea), Wulingsan (WLS, in China) and Goreisan (GRS, in Japan), are composed of the same five medicinal herbs: *Alisma orientalis* Juzep, *Poria cocos* Wolf, *Atractylodes macrocephala* Koidez, *Polyporus umbellatus* Fries, and *Cinnamomum cassia* Presl. The ratio of the component herbs is 5:3:3:3:2 in weight for WLS. The ratio is 5:3:3:3:1 for formula ORS from traditional Korean medicine book, Donguibogam. The ratio is 6:4:4:4:3 in weight for GRS (https://kampo.ca/herbs-formulas/formulas/goreisan/). Although the ratios of the component herbs are slightly different in the areas, reported effects of ORS, WLS and GRS are largely similar in the field of our discussion here.

The formula Wulingsan was first appeared in the traditional Chinese Medicine book, Shanghanlun (Treatise on Febrile Diseases) written by Zhang ZhongJing in the third century. The formula Oryeongsan appeared in the Donguibogam, “Treasured Mirror of Eastern Medicine” written by Heo Jun in the early seventeenth century in Korea. ORS, WLS and GRS have been known to possess curative effects in human subjects in the field of diseases including nephrolithiasis,[1,2] chronic subdural hematoma[3-5] and fluid imbalance (the present review).[6] The purpose of the present study is to review the recent opinions on the effects of ORS in the excretory function of the kidney, atrial natriuretic peptide hormone (ANP) secretion, and the regulation of the blood pressure

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homeostasis in particular and action mechanisms involved. Actions of ORS are associated with modulation of glomerular filtration rate (GFR), Na^+-H^+-exchanger isoform 3 (NHE3), vasopressin subtype 2 receptor (V_2R)/aquaporin2 water channel (AQP2) system in the kidney and ANP secretion through mechanochemical signaling in the atria via the cardio-renal hormone systems, renin-angiotensin system (RAS) and natriuretic peptide system (NPS), in hypertensive animal models.

2. Methods

The information discussed in this review was searched online using search databases including PubMed-NCBI and Google-Scholar.

3. Cardio-renal effects of Oryeongsan (Wulingsan)

3.1. *ORS increases urinary volume and excretion of Na^+*

ORS increases urinary volume and excretion of Na^+ through inhibition of NHE3 and V_2R/AQP2 signaling pathways in association with modulation of the RAS and NPS in the cortex and medulla of the kidney [tory on the urinary flow (UV), urinary excretion of Na^+ ($U_{Na}V$), body fluid and Na^+ balance-antihypertensive effect]. Previously, it was shown that an intravenous injection of ORS water extract (equivalent to dried ORS crude powder 87 mg/kg body weight) increased UV and excretion of electrolytes including Na^+ and Cl^- in rabbits anesthetized with thiopental sodium.[6] ORS-induced diuresis and natriuresis were accompanied by an increase in creatinine clearance (GFR) without significant changes in fractional excretion of Na^+ (FE_{Na}%). It was further observed that the treatment with ORS (0.1 ~ 10 mg/kg body weight/day orally for 1 week) increased UV, UNaV, U_KV and $U_{Cl}V$, and contraction of the body fluid and Na^+ balance along with suppression of the plasma levels of renin activity (PRA) and aldosterone (ALDO) in rats.[7] ORS increased UV with a decreased urinary osmolality (*vide infra*). These findings suggest that ORS-induced natriuresis, diuresis and an increase in GFR are associated with modulation of the RAS.

Recently, it was further found that chronic treatment with ORS (100 mg/kg/day orally) suppressed abundance of NHE3 expression in the cortex of the kidney along with natriuresis and diuresis in the Goldblatt model of renovascular hypertensive rats (Goldblatt hypertensive rats) and spontaneously hypertensive rats (SHR).[8,9] NHE3 is the major system involved in the regulation of the renal proximal tubule reabsorption of the filtered Na^+ (more than 60 % of the glomerular filtrate) in the cortex of the kidney.[10,11] Abundance of NHE3 expression was significantly suppressed in the cortex of the kidney from Goldblatt hypertensive rats compared to Sham rats.[8] The suppression was similarly observed in both of clipped and non-clipped kidneys. In contrast, in SHR, abundance of NHE3 gene expression was accentuated compared to that of WKY.[9] Chronic treatment with ORS significantly suppressed abundance of NHE3 gene expression in the cortex of the kidney, where the renal proximal tubules are located, from both models of hypertension.[8,9] ORS suppressed abundance of NHE3 expression in association with an increase in Angiotensin II subtype 2 receptor/Angiotensin converting enzyme 2-Mas receptor (AT_2R/ACE2-MasR) signaling pathways and concurrent decrease in Angiotensin converting enzyme-Angiotensin II subtype 1 receptor (ACE-AT_1R) expression in the renal cortex (Goldblatt hypertensive rats and SHR) and medulla (in Goldblatt; not tested in SHR) (Fig. 1). Recently, it has been shown that AT_2R activation with a selective agonist Compound-21 (C-21) induced natriuresis via internalization and inactivation of NHE3 in association with protein phosphatase PP2A subunits binding to AT_2R physically in the renal proximal tubule cells from WKY[12-14] (*vide infra*). This signaling pathway of the AT_2R is known to be defective in SHR.[12-15] ORS-induced suppression of NHE3 expression and accentuation of Na^+ excretion was accompanied by an increase in AT_2R expression in the kidney from Goldblatt hypertensive rats and SHR.[8,9] It has been shown clearly that AT_1R is localized mainly in the renal proximal tubule brush border and basolateral membranes, and then, distal tubules, and cortical and medullary collecting ducts.[16] The molecular mechanism by which ORS controls the expression of NHE3 in the kidney is not clear at present.

ORS-induced increase in urinary volume was characterized by a decrease in osmolality with suppression of the expression of V_2R/AQP2 signaling pathway in the medulla of the kidney from Goldblatt hypertensive rats[8] (Fig. 1) (*vide infra*). These are consistent with the previous reports on the regulation of urinary osmolality (urine concentration) via AQP2 signaling in association with modulation of AT_1R expression.[17-19] V_2R/AQP2 water channel signaling pathway in the medulla (collecting duct) of the kidney is the main site for the regulation of water reabsorption.

ORS-induced accentuation of GFR and renal excretory function, and suppression of the NHE3 and V_2R/AQP2 expression in hypertensive rats were closely associated with modulation of the NPS as well as the RAS expression in the kidney.[8,9] Treatment with ORS induced an increase in GFR in rabbits[6] and normotensive or hypertensive rats.[7-9] ORS-induced increase in GFR was accompanied by a decrease in the plasma levels of renin activity (PRA) and an increase in the plasma levels of ANP along with a suppression of AT_1R expression and an accentuation of AT_2R in the kidney.[8] Further, ORS induced a suppression of intra-renal renin synthesis and contents and an accentuation of ANP gene expression and a suppression of the ANP-clearing NPR-C expression in the kidney which expected to further increase intra-renal ANP concentration.[9] ANP dilates preglomerular arterioles and constricts glomerular efferent arterioles[20-22] leading to an increase in GFR. Glomerular afferent and efferent arterioles express selective receptors for ANP to increase GFR (afferent arterioles to be dilated and efferent constricted).[22] It has also been known that intra-renal blockade of AT_1R signaling pathway increases GFR.[23,24] Increase in GFR contributes to induce natriuresis.[25] Further, chronic treatment with ORS accentuated ANP secretion in the cardiac atria from SHR[26] which is associated with antihypertensive effects of ORS administration in hypertensive animals (Fig. 1). ANP induces an increase in GFR, natriuresis, diuresis, and vasodilation.[27]

Treatment with ORS induces an increase in urinary volume and excretion of Na^+ through an accentuation of GFR and atrial ANP secretion, and suppression of NHE3 and V_2R/AQP2 expression in the kidney, and results in a contraction of the body fluid and Na^+ balance leading to a decrease in high blood pressure in (primary or secondary) hypertensive rats. These are closely associated with modulation of the intra-renal RAS and NPS (Figs. 1 and 2).

3.2. *ORS modulates the RAS in the cardio-renal system*

ORS modulates the RAS in the cardio-renal system through suppression of ACE-AT_1R pathway and concurrent accentuation of AT_2R/ACE2-MasR signaling along with suppression of the renin synthesis. ORS/ WLS has been known to modulate the RAS.[7-9,26,28,29] WLS (480 mg/kg/day orally for 4 weeks) suppressed the adriamycin-induced increase in Ang II contents in the renal cortex from rats treated with adriamycin along with an increase in urinary volume.[28] Similarly, chronic treatment with Wuling Powder (WLS water extract, high, medium and low doses, for 8 weeks) decreased high blood pressure with suppression of plasma levels of renin activity, Ang II, and ALDO, and modulated the gene expression (suppression of AT_1R expression and accentuation of ACE2) in the cardiac muscle (cardiac apex) from SHR.[29] Further, treatment with ORS decreased PRA and intra-renal renin synthesis and modulated the RAS expression in the kidney or atria from normotensive and hypertensive rats.[7-9] ORS decreased intra-renal (cortex and medulla) renin synthesis and contents in the clipped kidney from Goldblatt hypertensive rats.[8] Also, ORS suppressed intra-renal ACE-AT_1R expression, and accentuated AT_2R and ACE2-MasR expression in Goldblatt hypertensive rats and SHR.[8,9] Further, ORS increased atrial secretion of ANP in perfused beating atria from SHR via modulation of the RAS: accentuation of AT_1R expression was associated with a suppression of ANP secretion,

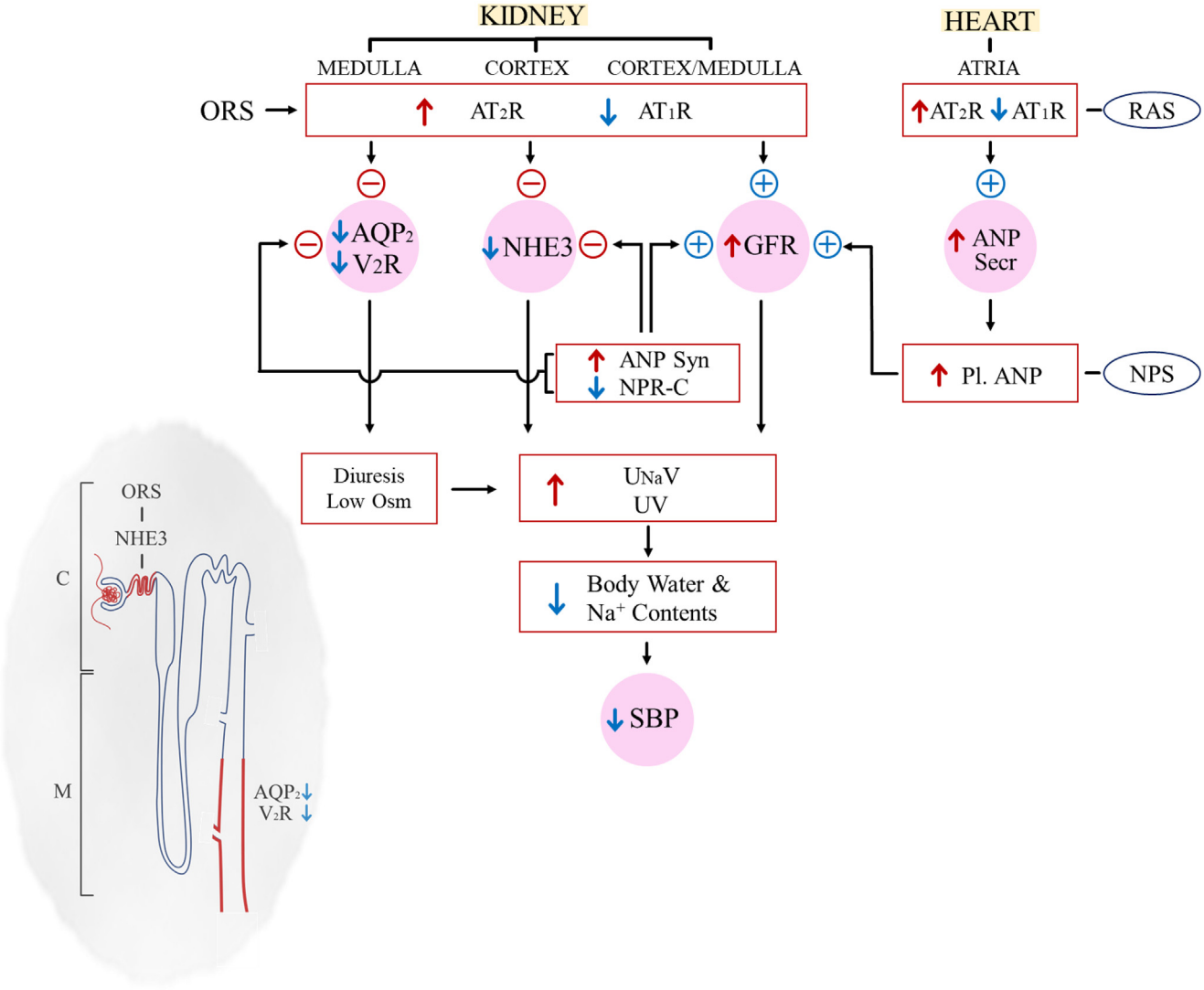

Fig. 1. Treatment with ORS increased UV and $U_{Na}V$ along with an increase in GFR and suppression of NHE3 and V_2R/AQP2 signaling pathway in the renal cortex and medulla, respectively, and accentuated ANP secretion in the atria. These processes were accompanied by an accentuation of AT_2R and concurrent suppression of AT_1R expression in the kidney and atria. Intrarenal ANP is also involved in the regulation of GFR, NHE3 and AQP2/V_2R signaling. RAS, Renin-angiotensin system; AT_2R, AT_2 receptor; NPS, Natriuretic peptide system; Pl, Plasma level; ANP Syn, Atrial natriuretic peptide synthesis; NPR-C; Natriuretic peptide receptor-C; Low Osm, Low osmolality; +, Accentuation; -, Suppression; ↑, Increase; ↓, Decrease; SBP, Systolic blood pressure; C, Cortex; M, Medulla.

while AT_2R was with an accentuation of ANP secretion.[26] These are consistent with the previous reports. Previously, it was shown that an activation of AT_1R with Ang II is associated with a suppression of ANP secretion in perfused beating atria from Goldblatt hypertensive rats,[30] while activation of AT_2R with Ang III is an accentuation of the hormone secretion in the atria from normotensive rats.[31]

ORS affected the regulation of body fluid and Na^+ balance homeostasis and vascular function via modulation of the RAS and NPS. Activation of AT_1R is for anti-diuresis and anti-natriuresis in the kidney; decrease in ANP secretion in the atria; impaired vasodilation: while activation of AT_2R/ACE2-MasR is for diuresis and natriuresis; increase in ANP secretion in the atria and amelioration of the impaired vasodilation.[8,9,26]

3.3. ORS suppresses NHE3 expression

ORS suppresses NHE3 expression through modulation of the RAS and NPS in the renal cortex. ORS induced an increase in UV and $U_{Na}V$ and contraction of the body water and Na^+ balance.[8,9] The process was closely associated with a suppression of NHE3 expression along with modulation of the RAS and NPS in the cortex of the kidney where the renal proximal tubules are located (Figs. 1 and 2). Chronic treatment with ORS increased UV and $U_{Na}V$ with a decrease in PRA in normotensive or hypertensive rats.[7-9] Further, the treatment with ORS suppressed intrarenal renin contents and its gene expression in the renal cortex from Goldblatt hypertensive rats and SHR.[8,9] Intra-renal effects of ORS on the RAS and NHE3 were similar in both of Goldblatt hypertensive rats and SHR. Activation of ACE-AT_1R pathway was associated with anti-natriuresis via an increase of NHE3 expression while that of AT_2R/ACE2-MasR was natriuresis via decrease of NHE3 signaling.

NPS is also directly involved in the regulation of NHE3 function in the cortex of the kidney. ANP administration inhibits Na^+-coupled antiport of the NHE3 in the renal proximal tubules.[32-34] In addition, in human proximal convoluted tubules, corin, a protease responsible for conversion of pro-ANP to ANP, pro-ANP/ANP and natriuretic peptide receptor (NPR)-A protein and mRNA are expressed.[35] ANP is found together with Corin. Corin protein presents in the apical membrane of the proximal convoluted tubules and ANP degrading protease neprilysin is abundant in the brush border of the same segment. Further, Corin is

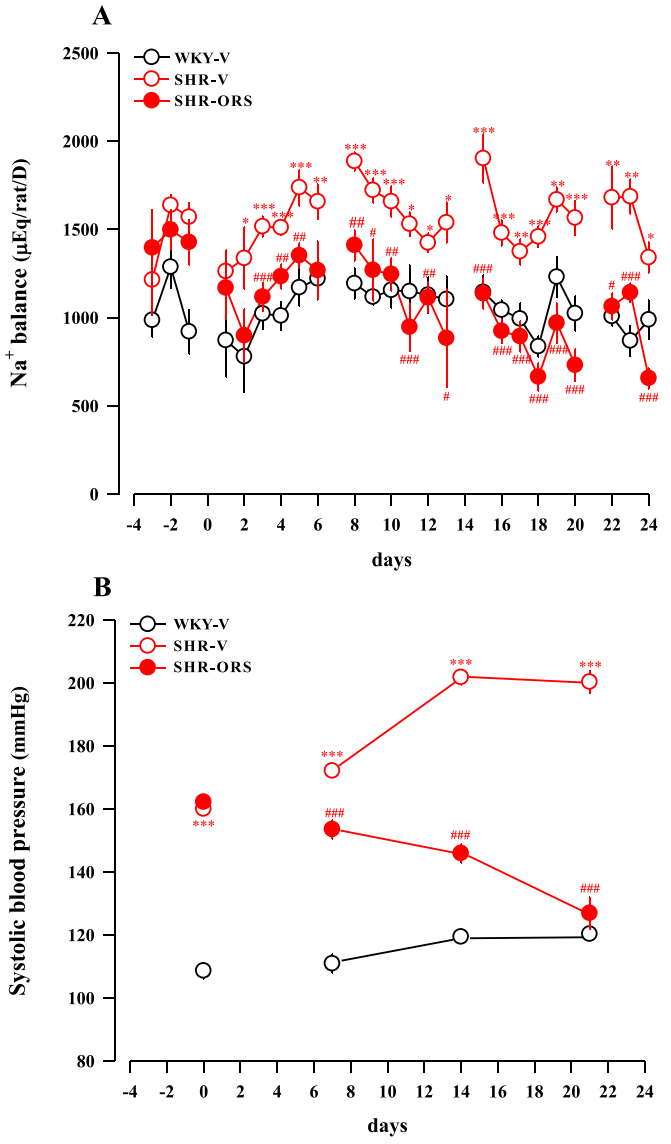

Fig. 2. A, Body Na^+ balance was significantly higher in the hypertensive SHR-V group treated with vehicle compared to normotensive WKY-V group treated with vehicle (Fig. 2A). SHR-ORS group treated with ORS increased urinary volume and excretion of Na^+ leading to a contraction of body water and Na^+ balance close to the levels of those of WKY-V (Fig. 2A). ORS induced contraction of body (water and) Na^+ balance resulted in a reduction of high blood pressure in SHR-ORS (Fig. 2A and 2B). B, SBP continuously increased in SHR-V group. ORS significantly decreased SBP in SHR-ORS group. On the days of blood pressure measurement no urine samples were collected. SHR-V, SHR group treated with vehicle; SHR-ORS, SHR group treated with Oryeongsan; *, **, ***, $P < 0.05$, $P < 0.01$, $P < 0.001$ vs WKY-V; #, ##, ###, $P < 0.05$, $P < 0.01$, $P < 0.001$ vs SHR-V. Fig. 2 is modified from Figs. 1E and 4 from Ahn et al., 2024 (Ahn et al., Integr Med RES. 2024;13:101,007).

also found in the proximal tubules of rat kidney.[36] In this occasion, the control levels of ANP is 5.6 ± 0.1 ng/mg tissue protein. Because the treatment with ORS accentuated ANP synthesis and suppressed ANP clearing natriuretic peptide receptor (NPR)-C in the renal cortex,[9] the concentration of ANP is expected to be further increased in the surrounding ISF in the cortex of the kidney (Fig. 1). One of the roles of NPR-C is to remove circulating natriuretic peptides from the circulation.[37] These findings suggest that ORS modulates intrarenal autocrine mechanism of ANP in the regulation of volume and pressure homeostasis through NHE3. This notion indicates that the NPS-NHE3 signaling could be a pathway affected by a treatment with ORS (Fig. 1). Increased ANP concentration in the renal cortex is expected to increase $U_{Na}V$ and UV through an inhibition of NHE3 activity. These findings show that ORS regulates NHE3 function through modulation of the systemic and intra-renal expression of the RAS and NPS.

3.4. ORS increases urinary volume with a decreased osmolality

ORS increases urinary volume with a decreased osmolality in association with a suppression of V_2R and AQP2 expression in the medulla of the kidney. Treatment with GRS water extract (100 or 300 mg/kg/day for 3 days orally) induced diuresis with a suppression of AQP2 mRNA expression in the cortex and medulla of the kidney from rats.[38] In this occasion, GRS had no significant effect on the V_2R expression in the kidney. It was further shown that the treatment with ORS (0.1 ~ 10.0 mg/kg/day orally for 7 days) induced natriuresis and diuresis with a decreased osmolality in rats.[7] Also, in Goldblatt hypertensive rats, chronic treatment with ORS (100 mg/kg/day orally for 3 weeks) induced natriuresis and diuresis with a decreased osmolality along with a suppression of the levels of V_2R and AQP2 expression in the renal medulla[8] (Fig. 1). These findings were associated with a suppression of ACE-AT_1R signaling pathway and concurrent accentuation of AT_2R/ACE2-MasR pathways in the renal medulla as well as in the cortex.

It has also been shown that expression of AQP2 gene expression and its function in the medulla of the kidney is closely associated with modulation by changes in the NPS as well as the RAS[39] (*vide supra*) (Fig. 1). ANP is inhibitory on the V_2R/AQP2 signaling in collecting duct principal cells.[39] ANP inhibits AVP-induced changes in water permeability through suppression of the AVP-dependent trafficking of AQP2 to the plasma membrane of collecting duct principal cells with decreased S256 phosphorylation via NPR-A/cGMP/PKG signaling pathway.

Further, ORS attenuated the hypertonic stress-induced increase in AQP2 protein expression and its apical membrane insertion in the cultured inner medullary collecting duct cell line (mIMCD-3).[40]

These findings show that the treatment with ORS/WLS/GRS induces natriuresis and diuresis with a decreased urinary osmolality accompanied by a suppression of the V_2R/AQP2 signaling pathway along with a modulation of the intra-renal RAS and NPS (Fig. 1).

3.5. ORS accentuates ANP secretion in the atria

Treatment with ORS accentuates ANP secretion in the atria from SHR via modulation of the RAS and M_2 mAChR-$K^+{}_{ACh}$ channel signaling in which the secretion is suppressed. Both the RAS and NPS are involved in the regulation of the body fluid and blood pressure homeostasis through the cardio-renal system. Treatment with Wuling powder[41] or ORS[8] induced an increase in plasma levels of ANP in normotensive mice or Goldblatt hypertensive rats, respectively. Further, treatment with ORS accentuated ANP secretion in the atria from SHR in which the secretion was suppressed.[26] ANP is synthesized in the cardiomyocytes of the atria and stored as secretory granules in the myocytes. The stored ANP is released into the interstitial space (interstitial fluid, ISF) of the atria in response to stretch of the cardiomyocytes during atrial distension and then followed by translocation of the released ANP to the atrial lumen (circulation) by atrial contraction.[42-44] Therefore, atrial secretion of ANP is accomplished by a "two-steps of sequential mechanism."[45] The first step cardiomyocyte release of ANP to the ISF surrounding the cardiomyocytes by atrial distension (stretch-induced release of ANP from the cardiomyocytes)[46] is followed by translocation of the released ANP with the ISF to the atrial lumen (atrial secretion of ANP) by contraction of the atria.[45] Plasma levels of ANP are controlled by atrial secretion of ANP and degradation by enzymes and clearing of the circulating hormone by natriuretic peptide receptor (NPR)-C located at the plasma membrane.[37,47] Atrial distension is main and the most important stimulus for the stimulation of ANP secretion.[44,48-50]

ACh is another secretagogue stimulating ANP secretion in the atria.[26,51,52] It was further shown that endogenous ACh is involved positively in the regulation of ANP secretion in the atria from rats.[52] ACh stimulates ANP secretion via activation of the M_2 muscarinic (m) ACh receptor-$G_{i/o}$-K^+_{ACh} (M_2 mAChR-K^+_{ACh}) channel signaling pathway in perfused beating atria.[26,51,52]

Stepwise increase in atrial distension elevated atrial ANP secretion proportionally in perfused beating atria from normotensive WKY. However, the ANP response induced by atrial distension was severely impaired in the atria from SHR compared to that in the atria from WKY.[26] Impaired ANP response of the atria was accompanied by an accentuation of abundance of AT_1R expression and concurrent suppression of AT_2R, and M_2 mAChR and G protein-coupled inwardly rectifying K^+ channel (GIRK4), a molecular component of K^+_{ACh} channel in the atria from SHR compared to WKY.[26]

Chronic treatment with ORS or losartan, an AT_1R blocker used as a positive control for ORS, reversed the suppression of ANP secretion to an accentuation of the atrial secretion of ANP, that is, an increase in cardiomyocyte release of ANP, in perfused atria from SHR in response to atrial distension or ACh administration.[26,53] Further, the treatment with ORS (or losartan) suppressed abundance of gene expression of AT_1R and accentuated AT_2R/Mas R and M_2 mAChR-GIRK4 signaling pathway in the atria compared to that in the atria from SHR treated with vehicle. Treatment with ORS or losartan reversed the impaired atrial ANP response to atrial distension or ACh administration with a reciprocal relation of the expression of the M_2 mAChR/GIRK4 to AT_1R in the atria from SHR[26] or Goldblatt hypertensive rats.[53]

Similarly, atrial distension- or ACh-induced activation of atrial ANP secretion was also suppressed but was reversed by treatment with losartan in perfused atria from Goldblatt hypertensive rats.[53] The suppressed response of ANP secretion was accompanied by an accentuation of AT_1R expression and concurrent suppression of AT_2R and M_2 mAChR-K^+_{ACh} channel signaling pathway in the atria from Goldblatt hypertensive rats[53] and SHR.[26] Previously, it was shown that Ang II-AT_1R signaling is associated with a suppression of ANP secretion in the perfused beating atria from Goldblatt hypertensive rats,[30] while AT_2R activation with Ang III, a selective AT_2R agonist, is associated with an accentuation of ANP secretion in the atria from normotensive rats.[31] These findings show that atrial distension- or ACh-induced activation of atrial ANP secretion is suppressed in animal models of hypertension.[26,30,53] Further, impaired ANP secretion is closely associated with an accentuation of AT_1R expression and concurrent suppression of the AT_2R in the atria.[26,53] AT_1R blockade with losartan reversed the suppression of ANP secretion with reversion of AT_1R and AT_2R expression in the atria from hypertensive rats.[26,30,31,53]

Treatment with ORS reversed the impaired ANP response to the atrial distension or ACh administration in the atria from SHR. Expression of the M_2 mAChR-K^+_{ACh} channel signaling pathway was suppressed along with an accentuation of AT_1R and concurrent suppression of AT_2R in the atria from SHR treated with vehicle but reversed by treatment with ORS.[26]

ANP secretion activated by atrial distension or ACh administration is impaired in the atria from hypertensive animal models.[26,30,31,53] Impaired ANP secretion is associated with an accentuation of AT_1R and concurrent suppression of AT_2R and M_2 mAChR signaling pathway in the atria from hypertensive rats.[26,53] Treatment with ORS accentuated atrial distension- or ACh-induced increase in ANP secretion along with suppression of AT_1R expression and concurrent accentuation of AT_2R and M_2 mAChR-K^+_{ACh} channel signaling pathway in the atria from hypertensive rats.[26]

3.6. Effects of component herbs of the ORS formula on the renal excretory function

The component herbs of the ORS formula affects the renal excretory function via V_2R/AQP2 signaling pathway. Oral intake of *Alisma orientalis* Juzep ethanol extract (0.9–4.0 g/kg body wt, orally) induced diuresis and natriuresis in rats.[54] The renal effects of *Alisma orientalis* Juzep were accompanied by decreased levels of AQP2 expression in rat renal medulla. Treatment with the extract also decreased AQP2 expression in human renal tubule epithelial cell line HK-2 cells.

Poria cocos water extract inhibited the hypertonic stress-induced increase in AQP2 water channel expression in the inner medullary collecting duct cell culture (mIMCD-3) model.[55] In addition, *Poria cocos* suppressed the puromycin-induced increase in AQP2 expression in the medulla of the kidney.[56] Further, oral administration of *Poria cocos* water extract (0.6 - 2.4 g/kg/day orally) induced diuresis with a decreased urinary osmolality accompanied by an inhibition of V_2R and AQP2 expression and reduced plasma levels of arginine vasopressin (AVP) in rats with chronic heart failure induced by acute myocardial infarction.[57]

Atractylodes macrocephala Koidez inhibits hypertonicity-induced increase in AQP2 water channel and its trafficking into the plasma membrane in the mouse inner medullary collecting duct (mIMCD-3) cell culture in the range of non-cytotoxic concentrations.[58]

A component herbal medicine of the ORS formula, *Polyporus umbellatus* Fries (zhuling) water extract showed similar effects to ORS formula on the diuresis and natriuresis.[59] Treatment with water extract of *Polyporus umbellatus* Fries (50 ~ 500 mg/kg BW/day orally for 8 days) induced diuresis and natriuresis along with suppression of V_2R and AQP2 gene expression in the medulla of the kidney from rats.

A major portion of the five medicinal herbs of the ORS formula induced diuresis and/or natriuresis with a suppression of V_2R/AQP2 expression in rats, mice, or cultured cells.

It is worthwhile to test effects of chemical components of the component herbs of ORS formula. Wang et al. investigated effects of *Polyporus umbellatus* and its main bioactive component ergone in the renal failure model of rats.[60] Serum level of creatinine, an indicator of the renal function, increased in the renal failure. Treatment with *Polyporus umbellatus* or ergone improved impaired renal function and significantly decreased serum levels of creatinine.

Further, ergone, a marker component of Polyporus umbellatus, induced natriuresis by blocking mineralocorticoids in rats. .[61]

Recently, it was further found that 19 different kinds of chemical components are present in the ORS formula.[62]

3.7. ORS reduces high blood pressure

ORS reduces high blood pressure along with a decrease in abundance of NHE3 and V_2R/AQP2 expression and an increase in GFR and atrial ANP secretion via modulation of the RAS and NPS. Treatment with WLS (4.8 ~ 19.2 g/kg/day orally for 8 weeks) significantly decreased high blood pressure in SHR.[29] The effects were accompanied by a decrease in the plasma levels of renin activity, Ang II, and ALDO. WLS further decreased AT_1R gene expression in the myocardial tissue. These findings suggest that WLS decreases high blood pressure in association with suppression of the RAS in SHR. Similarly, treatment with ORS (200 mg/kg/day orally for 3 weeks) significantly decreased systolic blood pressure with a decrease in tubulointerstitial fibrosis in the kidney from SHR.[63]

The plasma levels of renin activity and ALDO were increased in Goldblatt hypertensive rats compared to those of Sham-operated rats.[8] The levels of renin contents and its gene expression in the clipped (left) kidney from Goldblatt hypertensive rats were higher than those from Sham rats, while those of non-clipped (right) kidney were suppressed compared to those of the (right) kidney of Sham rats treated with vehicle. AT_1R gene expression was accentuated in the renal cortex from hypertensive rats compared to those from sham rats treated with vehicle. Renal proximal tubules located at the cortex of the kidney are the major site for the reabsorption of the filtered Na^+. Chronic treatment with ORS decreased abundance of Na^+ transporter NHE3 expression located at the renal cortex[8,9] (*vide supra*). ORS further decreased expression of the water channel signaling pathway V_2R/AQP2 system located at the medulla

(collecting duct) of the kidney.[8] These effects by ORS were associated with a suppression of the expression of ACE-AT_1R signaling pathway and concurrent accentuation of ACE2-MasR and AT_2R expression in the cortex and medulla of the kidney. Similarly, treatment with ORS significantly reduced the high blood pressure as observed in SHR.[9] ORS increased UV and UNaV in SHR compared to SHR treated with vehicle. These changes resulted in a contraction of the body fluid and Na^+ balance leading to reduction of high blood pressure along with modulation of GFR, NHE3, and V2R/AQP2 signaling pathway in the kidney[8,9] (Figs. 1 and 2). ORS-induced suppression of the NHE3 expression in the renal proximal tubules and accentuation of renal excretory function are consistent with the previous reports on the roles of NHE3 involved in the regulation of proximal tubule function for the filtered Na^+ reabsorption, and maintenance of basal and high blood pressure.[64-67] The role of NHE3 in the proximal tubules of the kidney is essential in maintaining the basal levels of blood pressure and lowering of high blood pressure in animal models of hypertension.[65] Chronic treatment with ORS decreased high blood pressure through an increase in UV and $U_{Na}V$ via accentuation of GFR and suppression of abundance of NHE3 and V_2R/AQP2 expression. The process was conducted via modulation of both the RAS, AT_1R and AT_2R, and NPS signaling in the cortex and medulla of the kidney from animal models of hypertension (*vide supra*). These changes resulted in a contraction of the body water and Na^+ balance in the primary or secondary hypertensive rats[8,9] (Figs. 1 and 2). These findings support the previous report on the systems pharmacology approach showing importance of the role of the RAS to define the mechanisms of antihypertensive effects of Oryeongsan.[68]

In addition, the treatment with ORS ameliorated impaired vasodilation in SHR.[9] ACh-induced vasodilation was impaired in the isolated aorta strips from SHR compared to that from WKY. The impaired response of the aorta to ACh was accompanied by an accentuation of AT_1R gene expression and concurrent suppression of AT_2R. Chronic treatment with ORS ameliorated the impaired vasodilation and reversed the expression of AT_1R and AT_2R, that is, an accentuation of AT_2R and concurrent suppression of AT_1R in the aorta from SHR treated with ORS. Previously, it has been shown that vasodilation is impaired in the carotid artery strips in association with an accentuation of AT_1R expression.[69]

4. Conclusions

In summary, the action sites of ORS in association with the regulation of the body fluid and Na^+ balance and blood pressure homeostasis are the glomeruli and renal proximal tubules in which the major portion of the glomerular filtration is reabsorbed. The roles of the glomeruli and NHE3 in the renal proximal tubule, and V_2R/AQP2 in the collecting duct are involved in amelioration by ORS of the impaired regulation of the body fluid and Na^+ balance in hypertension via modulation of the RAS and NPS. This notion is in agreement with Guyton's proposal on the "long-term arterial pressure control" through "the kidney-fluid volume system" in the regulation of volume and pressure homeostasis of the body.[70] ORS/WLS/GRS-induced modulation of the RAS and NPS are involved in the regulation of body fluid and blood pressure homeostasis through the kidney directly or indirectly.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

CRediT authorship contribution statement

Ho Sub Lee: Conceptualization, Writing – review & editing, Project administration, Funding acquisition. **Hye Yoom Kim:** Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Data curation, Visualization, Funding acquisition. **You Mee Ahn:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft. **Kyung Woo Cho:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration.

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Ethical statement

Not applicable.

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Data availability

Data sharing is not applicable to the manuscript. No new data were included in the manuscript.

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