Lithium effectively complements vasopressin V2 receptor antagonist in the treatment of hyponatraemia of SIADH rats

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Abstract

Background. Although, pharmacological intervention with a selective arginine vasopressin (AVP) V2 receptor antagonist has been demonstrated to be effective for syndrome of inappropriate secretion of antidiuretic hormone (SIADH), its long-term administration has some therapeutic limitations. Lithium, a drug for bipolar disorders, has been known to cause nephrogenic diabetes insipidus by reducing kidney-specific apical water channel, aquaporin 2 (AQP2) expression in the collecting ducts. However, its pharmacological efficacy for SIADH still remains to be elucidated.

Methods. Hyponatraemia was induced in male Sprague–Dawley rats by water loading and subcutaneous infusion of 1-deamino-8-D-arginine vasopressin. For the treatment, lithium chloride (LiCl) was administered singly or in combination with OPC-31260 and/or furosemide for 7 days. Protein expression of AQP2 was examined by western blotting at the end of the observation period.

Results. The LiCl administration elevated serum sodium levels in a dose-dependent manner. The therapeutic effect started 3 days after the initial administration and gradually increased. Western blot analysis at the end of the treatment demonstrated dose-dependent reduction of AQP2 protein expression. Additional administration of LiCl (100 mg/kg/day, the dose demonstrated to maintain serum lithium concentration within therapeutic range) to low dose OPC-31260 maintained well the initial elevation of serum sodium level during the treatment. Western blot analysis after combination therapy demonstrated the absence of re-increase in AQP2 expression noted at the end of OPC-31260 treatment. However, further additive effect could not be obtained even when both LiCl and furosemide were added together to low dose OPC-31260.

Conclusions. Although the single effect of therapeutic dose of lithium was weak, it effectively and safely compensated for the therapeutic limitations of a low dose of AVP V2 receptor antagonist for SIADH by reducing AQP2 expression.

Keywords: adenylyl cyclase; aquaporin 2; combination therapy; lithium chloride; nephrogenic diabetes insipidus; OPC-31260

Introduction

Syndrome of inappropriate secretion of antidiuretic hormone (SIADH) is a condition in which plasma arginine vasopressin (AVP) level is not appropriately suppressed despite hypo-osmolality [1]. Sustained water intake due to chronic exposure to AVP can result in net water retention and the development of hyponatraemia [2]. Therefore, fluid restriction for ameliorating excessive water retention has been the mainstay of treatment of SIADH patients. However, the effect has been limited, in part, by the poor compliance of the patients. To compensate for such therapeutic limitation of fluid restriction, several pharmacological interventions have been tried, although they were not always effective [3–5]. Recently, Fujisawa et al. [6] demonstrated the
Lithium treatment for SIADH rats

therapeutic efficacy of an orally effective and highly selective non-peptide AVP V2 receptor antagonist, OPC-31260, for SIADH rats. The OPC-31260 was further demonstrated to reduce the enhanced expression of the kidney-specific apical collecting duct water channel, aquaporin 2 (AQP2), in SIADH rats [7]. In clinical trial, Saito et al. [8] demonstrated its substantial effect on improving hyponatraemia in SIADH patients. In our previous study, however, we have demonstrated in SIADH rats that the therapeutic effects of long-term OPC-31260 administration is limited, since the effects declined again with increasing number of treatment days and a high-dose administration induced severe toxicity [9]. Although AQP2 protein expression was reduced shortly after OPC-31260 administration, it increased again in parallel with the decline of therapeutic effects. Additional administration of loop diuretics, furosemide, successfully complemented such therapeutic limitation of OPC-31260. However, extremely high dose was required, indicating the difficulty in putting it into clinical practice.

Lithium is a drug used effectively for the treatment of manic-depressive disorders [10]. However, long-term use of lithium is frequently complicated by impaired water reabsorption, resulting in nephrogenic diabetes insipidus (NDI) [11]. In the 1970s, based on its mechanism of reducing the renal action of AVP [12], lithium had been used for the treatment of SIADH patients with successful outcomes [13–15]. However, because of its inconsistent results and significant side effects including central nervous system disorders, it was deemed impractical for clinical use and subsequently withdrawn [16,17], although its precise effects have not yet been demonstrated in animal models of SIADH. Therefore, questions of optimal dosage and toxicity have remained unsolved. The entry of lithium into the renal tubular cell and its accumulation in renal medulla occurs through sodium channels, sodium-proton exchangers and sodium transporters, which exhibit a high selectivity for Li+ ions as well as Na+ ions [12,18,19]. Recently, impairment of AVP-sensitive adenylate cyclase (AdC) and subsequent decrease in AQP2 protein expression in the cells of the renal collecting ducts have been demonstrated as one of the mechanisms of lithium-induced urinary concentrating defect [20–22]. Increased production of prostaglandin E2 (PGE2) by lithium has also been demonstrated to suppress AdC activity, and reduce adenosine 3',5'-cyclic monophosphate (cAMP) synthesis in rat renal medulla [23]. Since increased AQP2 expression has been demonstrated in SIADH rats [7,24,25], lithium was expected to show therapeutic effect in SIADH by reducing this protein expression. Therefore, the purpose of our study was to evaluate the effect of lithium on SIADH, and based on these results, we further investigated whether lithium could complement the effect of AVP V2 receptor antagonist or not. To this end, we administered lithium chloride (LiCl) singly, or in combination with OPC-31260 to SIADH rats and examined their effects on various physiological parameters together with the alteration of AQP2 protein expression. Our present study has provided novel insights into the pharmacological therapy of SIADH.

Methods

Experimental animals

Male Sprague-Dawley rats weighing 200 g were used. They were housed in individual cages in a humidity- and temperature-controlled room with a 12–12 h light-dark cycle. All experimental protocols described in the present study were approved by the Ethics Review Committee for Animal Experimentation of Tohoku University.

Animal preparation

A slightly modified version of the SIADH model originally developed by Verbalis [26] was used as described previously [9]. Briefly, rats were acclimated for 4 days to a gelled-agar diet composed of 67% water, 27% finely powdered rat chow, 4.7% glucose and 1.3% agar, in which 0.084% sodium and 0.27% potassium were contained. The agar was melted in boiling water and poured into a mould, and then the powdered chow and glucose were added and stirred to an even consistency. The diet was then chilled at 4°C to form a firm, gelatin-like state. The rats received 90 g of this preparation each day. The diet forced the rats to consume a greater volume of water to take enough calories. After 4 days of the acclimatization period with water loading, the SIADH rats underwent osmotic minipump (model 2002, Alza Corporation, Palo Alto, CA, USA) implantation. Osmotic minipumps containing 1-deamino-8-D-arginine vasopressin (dDAVP; Kyowa Hakko, Tokyo, Japan) dissolved in 0.15 mol/l sodium chloride (NaCl) to make a concentration of 10 µg/ml were implanted subcutaneously on the back under ether anaesthesia, and dDAVP was infused at a rate of 5 ng/h. After recovery, the animals were placed in cages with free access to the gelled-agar diet for the additional days. All the rats were given the same amount of the gelled-agar diet over the entire course of the experiment. They were placed individually in metabolic cages to facilitate urine collection and recording of individual food and water intake. Twenty-four hour urine collection was conducted daily in order to measure urine volume, urine osmolality. Body weight and water and food intake were also measured daily.

Therapeutic interventions

According to the groups listed below (n=6 per group), LiCl was administered singly, or in combination with OPC-31260 and/or furosemide to the SIADH rats: (i) administration of LiCl—in group 1, the SIADH model rats were administered vehicle for LiCl; in group 2, 50 mg/kg LiCl (Wako Pure Chemical Industries Ltd, Osaka, Japan); in group 3, 100 mg/kg; in group 4, 200 mg/kg and in group 5, 400 mg/kg; (ii) combined administration of OPC-31260 and LiCl—in group 6, the SIADH rats were administered 5 mg/kg OPC-31260 singly (Otsuka Pharmaceutical Co. Ltd, Tokushima, Japan) and in group 7, 5 mg/kg OPC-31260 and 100 mg/kg LiCl in combination; (iii) combined administration of OPC-31260, LiCl and furosemide—in group 8, the SIADH rats were administered 5 mg/kg OPC-31260, 100 mg/kg LiCl
and 30 mg/kg furosemide (Wako Pure Chemical Industries Ltd, Osaka, Japan) in combination and in group 9, 5 mg/kg OPC-31260, 100 mg/kg LiCl and 100 mg/kg furosemide were administered. All the SIADH rat groups were subjected to oral administration of the drugs via a gastric tube once a day from days 7 to 13 at a dose of 2.0 ml/kg. The drugs were suspended in water containing 1% methylcellulose (Wako Pure Chemicals, Japan). We sampled 0.3 ml of blood from the tail veins to determine serum sodium levels every day during the treatment. Serum creatinine and urea concentrations were determined before and after the entire course of treatment. For the removal of kidneys, 4 ml of trunk blood was collected by bleeding the animals to death under ether anaesthesia, before and 7 days after the initial administration of drugs. Then, kidney tissue was sampled and used in immunoblotting experiments.

Immunoblotting

Immunoblotting using a specific antibody against AQP2 was performed as previously described [9,27]. Briefly, after dividing the harvested kidneys into three parts: cortex, outer medulla and inner medulla, the renal inner medulla was homogenized in 2 ml of PBS, 1% Triton, 1% deoxycholate, 0.1% SDS and 0.1 mM phenylmethylsulfonyl fluoride for protein extraction. Initially, 25 μg of protein from each of the samples was loaded in each lane for Laemmli’s SDS-polyacrylamide gel electrophoresis (PAGE) (12.5%). The gel was stained with Coomassie Brilliant Blue (G250; BioRad) to assess the quality of protein by sharpness of the bands and to confirm the equality of loading. For immunoblotting, 25 μg of protein was loaded in each lane for Laemmli’s SDS-PAGE (12.5%), and then transferred to a polyvinylidene difluoride membrane. The membrane was blocked for 1 h and exposed to antibody diluted with 2.5% milk powder/TBST (10 mM Tris-HCl, pH 8.5, 150 mM NaCl and 0.1% Tween-20) overnight at 4°C, and then to a second antibody (peroxidase-linked anti-rabbit IgG) for 1 h at room temperature. After washing, antigen–antibody complexes were visualized with a chemiluminescence system (ECL Plus, Amersham Bioscience, Piscataway, NJ, USA).

Other measurements and statistical analyses

Serum sodium, potassium, urea and creatinine levels were measured by a chemical auto-analyser (DRI-CHEM 3500V, Fuji Film, Tokyo, Japan). Urinary osmolality was determined with an osmometer (model 3D2; Advanced Instruments, Needham Heights, MA, USA). The results are expressed as means ± SEM. Statistical comparisons in all the physiological and laboratory data were made among the treatment groups using analysis of variance (ANOVA) followed by Dunnett’s or Student’s t-test for individual comparison. A P-value < 0.05 was considered significant.

Results

Effects of LiCl on serum sodium levels

Administration of LiCl. In the previous reports, AQP2 expression has been demonstrated to be increased in SIADH rats [7,24,25]. Since the expression of AQP2 protein is promoted by AVP through V2 receptor and subsequent AdC-mediated pathways [28,29], lithium, which is known to inhibit AdC [20,22], was examined for the treatment of the SIADH rats. Oral administration of LiCl was started on day 7 when the physiological reactions to the dDAVP infusion were stabilized [9]. Figure 1A demonstrates the LiCl dose-dependent effects of serum sodium levels. The LiCl doses higher than 100 mg/kg started elevating serum sodium levels 3 days after the initial administration. All the rats that were administered 400 mg/kg LiCl showed such abnormal behaviour as agitation, hypersensitivity and tremor other than haematuria and diarrhoea. They all expired during the course of treatment. Although all the rats that were administered 200 mg/kg LiCl survived the observation period, two of them showed the abnormal behaviour described above and were in a debilitating condition at the end of the observation period. In rats that were administered 100 mg/kg LiCl, although the elevation of serum sodium level was much slower and smaller than that in rats that were administered higher dose of LiCl, the elevated serum
sodium level was well-maintained during the rest of the treatment days, and no abnormal behaviour was noted. Administration of <50 mg/kg LiCl did not show any therapeutic effects.

**Combined administration of OPC-31260 and LiCl.** In our previous study, although OPC-31260 abruptly elevated serum sodium level in SIADH rats, this therapeutic effect was gradually decreased with increasing number of treatment days [9]. On the other hand, in the present study, lithium was demonstrated to elevate serum sodium level at the late phase of the treatment. Therefore, we tested the effects of combination therapy with a low dose OPC-31260 and LiCl. Based on our results, a 100 mg/kg LiCl was used for combination therapy. As shown in Figure 1B, when 5 mg/kg OPC-31260 was administered in combination with LiCl, although the initial elevation of serum sodium level was the same with that of single administration of 5 mg/kg OPC-31260, the effect was well-maintained throughout the observation period.

**Combined administration of OPC-31260, LiCl and furosemide.** In our previous study, although combination of OPC-31260 and furosemide showed additive therapeutic effects on SIADH rats, extremely high dose furosemide was required to obtain such effects [9]. To examine whether we can reduce the required dose of furosemide by further addition of lithium, we then, tested the effects of combination therapy with a low dose OPC-31260, 100 mg/kg LiCl and 30 mg/kg furosemide. However, as shown in Figure 1B, no additive effect was obtained. On the contrary, the change in serum sodium level was about the same with that of single administration of 5 mg/kg OPC-31260 (Figure 1B). When 100 mg/kg furosemide was combined instead of 30 mg/kg, initially elevated serum sodium level was further elevated, and was well-maintained throughout the observation period (data not shown), which was the same with combination of a low dose OPC-31260 and 100 mg/kg furosemide demonstrated in our previous study [9].

**Physiological changes during treatments**

Whereas a high dose lithium demonstrated toxicity, combination of lithium and a low dose OPC-31260 did not show any side effects. To elucidate these differences, we closely analysed the physiological changes in the SIADH rats during the course of treatment.

**Changes in body weight.** Figure 2 show the changes in body weight of all the rats. The body weights of the rats that were administered LiCl are shown in Figure 2A. The rats that were administered <100 mg/kg LiCl continued to gain weight. Although most of the rats that were administered 200 mg/kg LiCl did not show significant weight loss (Figure 2A), two of them showed progressive weight loss (data not shown) and were in a debilitating condition at the end of the observation period. The rats that were administered 400 mg/kg LiCl showed further weight loss and all of them died during the course of treatment. The body weight of rats subjected to combination therapy (Figure 2B) did not show any abnormal weight loss and continued to gain weight.

**Changes in urine volume, urine osmolality and water balance.** Figure 3A and B show the time course of urine volume and urine osmolality, respectively. To further analyse body fluid condition, water balance was determined by subtracting urine volume from water intake, as shown in Figure 3C. We measured the parameters after administration of the drugs. In rats subjected to <200 mg/kg LiCl administration, no changes in any of these parameters were observed (Figure 3A–C). Although no data are shown, all rats that were administered 400 mg/kg LiCl showed severe decrease in water balance and expired during the observation period, indicating that a high dose of lithium may induce marked reduction of intake, resulting in severe dehydration and malnutrition. In rats subjected to combination therapy with 5 mg/kg OPC-31260, a rapid increase in urine volume
and a corresponding decrease in urine osmolality (Figure 3B) were noted 1 day after drug administration. These changes were transient and may be related to the initial elevation of serum sodium levels in combination therapy (Figure 1B). In these rats, no changes in water balance were observed (Figure 3C).

Serum levels of urea, creatinine and potassium. Serum levels of urea, creatinine and potassium were measured before and after drug administration in rats that were alive at the end of the observation period. The LiCl did not alter the serum levels of creatinine and potassium. Serum urea levels, however, were significantly ($P < 0.05$) elevated in rats that were administered 200 mg/kg LiCl (from 12.0 ± 0.43 to 19.1 ± 2.4 mg/dl), whereas it remained unchanged in rats that were administered <100 mg/kg LiCl (from 12.9 ± 1.1 to 13.2 ± 0.59 mg/dl and 12.8 ± 0.65 to 14.0 ± 0.49 mg/dl in rats that were administered 50 mg/kg and 100 mg/kg LiCl, respectively).

Changes in AQP2 protein expression after LiCl administration

The absence of difference in urinalysis among therapeutic groups in the present study indicates the gradual appearance of the effects of lithium, which is in contrast to the rapid appearance observed in OPC-31260 treatment in our previous study [9]. To further investigate the mechanisms underlying the late therapeutic effects of lithium, we examined AQP2 protein expression after 7 days of LiCl administration (Figure 4), when dose-dependent difference of therapeutic effects became most prominent (Figure 1A). Western blots demonstrated the dose-dependent reduction of AQP2 protein expression in rats that were administered 100 mg/kg and 200 mg/kg LiCl, compared with that before treatment (Figure 4A). Statistically significant difference between rats before and after 100 mg/kg LiCl treatment and between rats after 100 mg/kg and 200 mg/kg LiCl treatment was confirmed by measuring signal density (Figure 4B).

Changes in AQP2 protein expression after combination therapy

In our therapeutic intervention, although the combined treatment with OPC-31260 and LiCl showed additive therapeutic effect of increasing serum sodium level, such additive effect was totally extinguished by further addition of furosemide (Figure 1B). To investigate the underlying mechanisms of these combination therapies, we compared AQP2 protein expression after the treatment among groups with these combination therapies (Figure 5). The AQP2 protein expression increased 7 days after initial administration of 5 mg/kg OPC-31260 compared with that before treatment (data not shown), which was consistent with the results of our previous study [9]. In rats with combined administration of 5 mg/kg OPC-31260 and 100 mg/kg LiCl, AQP2 protein expression was reduced compared with that of OPC-31260 alone (Figure 5A). However, in rats with combined administration of 5 mg/kg OPC-31260, 100 mg/kg LiCl and 30 mg/kg furosemide, such reduction of AQP2 protein expression was absent (Figure 5A). Statistically significant difference between rats after OPC-31260 treatment and combination therapy of two drugs (OPC-31260 and LiCl) and between rats after combination therapy of two drugs and three drugs (OPC-31260, LiCl and furosemide) was confirmed by measuring signal density (Figure 5B).
Discussion

Lithium has been demonstrated to decrease AQP2 protein expression in normally hydrated Sprague–Dawley rats and cause NDI [21,22]. This drug is thought to inhibit AdC activity in V2 receptor-mediated pathways for AQP2 protein expression [20,22]. In the SIADH rats, since the high expression of AQP2 is thought to be induced by AVP stimulus and the subsequent AdC-mediated pathways [9], lithium, which blocks the effects of AdC, may reduce the expression of this cell surface protein, theoretically leading to the remission of the disease. As expected, this drug suppressed dDAVP-induced AQP2 expression, and elevated serum sodium levels in a dose-dependent manner. However, LiCl, as high a dose as 400 mg/kg showed severe toxicity including manifestations of central nervous system disorder as reported previously [16,17]. A dose of 200 mg/kg led to reduced water intake and severe reduction in body weights in two of six rats (data not shown), while doses of 100 mg/kg or less did not. Thus, 100 mg/kg was thought to be the upper limit for the therapeutic use of this drug. The therapeutic effect of 100 mg/kg LiCl gradually increased with increasing number of treatment days. However, the effect was so weak that they did not change urine osmolality and urine volume. Moreover, the effect did not start until 3 days after the initial administration. In the original reports of rats with lithium-induced polydypsia, at least 7 days of feeding lithium-containing diets were required to lower the urine osmolality [30,31]. In patients with SIADH, it also took 3 days for orally administered lithium carbonate to be effective [13–15]. Such time lag is thought to be derived from the gradual achievement to optimal serum concentration of lithium, when orally administered [32]. The rate of absorption will reach plateau within 12–24 h, and then, the drug will slowly distribute through all body fluids. Therefore, it was considered to take more than 48 h for lithium to produce pharmacological effects on animal models also in the present study.

In our previous study, although AVP V2 receptor antagonist, OPC-31260, showed dose-dependent therapeutic effects soon after the administration, higher doses more than 10 mg/kg showed severe toxicity [9], as was observed in more than 200 mg/kg LiCl administration, in the present study. Therefore, in our previous study, the low dose of 5 mg/kg was thought to be the upper limit for the therapeutic use of OPC-31260. However, the effects of a low dose of OPC-31260 were weak, and a gradual decrease of serum sodium level was noted with increasing number of treatment days. In parallel with the decline of therapeutic effects, gradual re-increase in total AQP2 protein expression was demonstrated by western blotting. Although, we have not assessed the apical AQP2 protein expression in the collecting ducts,
reversion of urine osmolality as well as sodium and urea accumulation in renal medulla, indicated the discontinuation of vasopressin escape [9]. From our results of the present study, lithium was expected to show additive therapeutic effect at the later phase of the treatment, and compensate for the possible discontinuation of vasopressin escape by OPC-31260. Thus, we designed a combination therapy using a low dose of OPC-31260 and LiCl. Based on the present finding that 100 mg/kg LiCl was the most effective dose without toxicity, we combined 100 mg/kg LiCl to 5 mg/kg OPC-31260. The results demonstrated the additive therapeutic effects with the maintenance of elevated serum sodium level during the treatment. Absence of significant differences in urine volume and water balance during the treatment indicated the absence of significant alteration in circulatory blood volume. Thus, combination therapy with OPC-31260 and LiCl effectively ameliorated hyponaemia without affecting the physiological conditions of rats, which was also demonstrated in combination therapy with OPC-31260 and furosemide, in our previous study [9].

In the present study, LiCl has been demonstrated to suppress dDAVP-induced AQP2 expression and ameliorated hyponaemia of SIADH rats (Figures 1A and 4). Then, to assess the underlying mechanisms of combination therapy with OPC-31260 and LiCl, we examined AQP2 protein expression after the treatment. AQP2 protein expression after 7 days of combination therapy showed the absence of re-enhancement noted after OPC-31260 treatment (Figure 5). This suggests that, in combination therapy, the acute reduction of AQP2 protein expression by a low dose of OPC-31260 leads to a rapid and marked elevation of serum sodium level in the short term, after which lithium may work gradually at the late phase to compensate for the effect of OPC-31260 by reducing the re-enhanced AQP2 protein expression. Since both of these drugs could be combined without causing any side effects, this combination therapy was considered to be useful for the treatment of SIADH. Oral administration of 4 mmol/kg/day LiCl, which corresponds to 170 mg/kg/day, has been demonstrated in earlier experiments to maintain serum sodium levels within therapeutic ranges in rats [32]. The lower dose of LiCl (100 mg/kg) used in the present study was thought not to exceed the therapeutic range. In addition, 100 mg/kg lithium for rats has also not been shown to lead to morphological changes, as judged by light or electron microscopy [33,34]. These indicate, at least, that 100 mg/kg LiCl is not an extremely high dose for rats. Our present study, then, demonstrated that this therapeutic dose of lithium could complement the effect of low dose V2 receptor antagonist on SIADH.

For renal water reabsorption through AQP2, sodium reabsorption is required in advance to induce an osmotic gradient in the renal medulla. A kidney-specific sodium cotransporter (BSC1; bumetanide-sensitive cotransporter) in the thick ascending limb of Henle (TAL) supplies critical sodium to the medullary interstitium for concentrating urine [35,36]. We have demonstrated the alteration of BSC1 protein expression in various pathological conditions. Increased rat BSC1 (rBSC1) expression led to water retention in rats with cardiac infarction [37,38], while decreased rBSC1 expression led to urinary concentrating defect in rats with chronic renal failure [39]. In our previous study, increased rBSC1 protein expression was noted in the SIADH rats, and it was further increased after long-term OPC-31260 administration, in parallel with the decline of therapeutic effects [9]. To compensate for such decline of therapeutic effects, in part, by increased rBSC1 expression, furosemide, which limits the rBSC1 function, was added to OPC-31260 in our previous study. However, extremely high dose (100 mg/kg) furosemide was required for the compensation, and lower dose, such as 30 mg/kg, was not enough to show significantly additive therapeutic effect. Therefore, lithium was considered to be superior to loop diuretics in compensating for the therapeutic limitations of AVP V2 receptor antagonist, since therapeutic dose of LiCl compensated for the effects of OPC-31260 as mentioned before.

Finally, expecting the further additive effect and reduction of each required dose, we attempted combined therapy of OPC-31260, furosemide and LiCl, each of which has already been demonstrated to show therapeutic effects for SIADH. However, the additive therapeutic effect obtained by combination of OPC-31260 and LiCl was totally extinguished by further addition of 30 mg/kg furosemide (Figure 1B). Western blot analysis demonstrated the retrieval of increased AQP2 protein expression after the treatment, compared with that after the combination of OPC-31260 and LiCl (Figure 5). Some possibilities regarding the retrieval of AQP2 expression by the addition of furosemide were considered. First, chronic administration of furosemide-induced hypovolaemia or stimulated aldosterone secretion, which could increase AQP2 protein expression [28,40]. Second, lithium has been demonstrated to enter into renal medulla through sodium transporters [12] and suppress AdC activity in cells of the renal collecting ducts to reduce AQP2 expression [20–23]. Our previous study has demonstrated that furosemide functionally inhibits BSC1 to block lithium entry into renal medulla [22]. Therefore, in the present study, although lithium entry was thought to have been accelerated through increased BSC1 by OPC-31260 as demonstrated in our previous study [9], 30 mg/kg furosemide was considered to be enough to block such increased lithium entry through BSC1, resulting in the retrieval of AQP2 expression. Third, while lithium stimulates the medullary interstitial cells to increase renal PGE2 production and reduce AQP2 expression [41], furosemide inhibits medullary PGE2 production through reduced interstitial tonicity [42]. Thus, AQP2 expression was thought to have been retrieved by the addition of furosemide. Otherwise, an extremely high dose of furosemide (100 mg/kg) was required again to obtain the additive effect, although it was not more than the effect of combination of OPC-31260 and furosemide in our
previous study [9]. Thus, combination of OPC-31260, furosemide and LiCl was not thought to be useful for the treatment of SIADH.

In conclusion, we have demonstrated the therapeutic efficacy of lithium in complementing the effect of V2 receptor antagonist for SIADH, by reducing the re-enhanced AQP2 protein expression. Since both of these drugs could be combined in therapeutic doses without causing any side effects, this combination therapy was considered to be useful for the treatment of SIADH. However, further additive effect could not be obtained by further addition of loop diuretics, possibly due to the counteraction between lithium and loop diuretics.

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