Original article

Development of a young rat hyperkalemia model with decreased renal excretion of potassium

Running title: Rat K+ excretion-reduced hyperkalemia

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19 Number of text pages: 35

20 Figures: 5

21 Table: 2

22 Grants: This study was supported by Dokkyo Medical University, Young Investigator

23 Award (Grant No. 2021-09).

24

Abstract

Infants and young children are prone to hyperkalemia caused by decreased renal potassium excretion. However, there are limitations to the use of current hyperkalemia drugs in pediatric patients. To address this issue, we developed a young rat hyperkalemia model by the combined administration of potassium chloride (KCl) and amiloride (Ami) to test new drug candidates for young patients. We administered KCl (2, 3, 4.5, and 6 mEq/kg) and Ami (3 mg/kg) intraperitoneally to five-week-old male Wistar-Imamichi rats and placed them in metabolic cages. After 6 h, blood and urine samples were collected. The combined administration of KCl and Ami increased the serum-potassium concentration compared to the control (2 mEq/kg; 5.2 vs 4.5 mEq/L, $P = 0.039$, 6 mEq/kg; 6.8 vs 4.1 mEq/L, $P=0.005$, n=5) in a dose-dependent manner. Next, the rats were orally administered furosemide (30 mg/kg, FURO), a known hyperkalemia treatment, or Compound 21 (C21) (10 and 30 mg/kg), an angiotensin II type 2 receptor agonist and candidate drug for hyperkalemia. FURO was administered as a single dose (after 1 h) or as repeated doses (after 1, 3, and 5 h). The serum-potassium concentration was significantly decreased by FURO (single dose; 5.9 vs 6.8 mEq/L, $P=0.017$, n=7, repeated doses; 5.6 vs 6.3 mEq/L, $P=0.021$, n=7). However, C21 did not decrease the serum-potassium concentration compared to the control (6.6 vs 6.8 vs 7.2 mEq/L, $P=0.07$).

Overall, this model can be used to evaluate the effects of candidate therapeutic drugs for hyperkalemia in children caused by decreased urinary-potassium excretion.

Amiloride, Angiotensin II type 2 receptor agonist, Compound 21, Pediatrics, Renal hyperkalemia

Introduction

Hyperkalemia is defined as a serum or plasma potassium level that is higher than the upper limit of normal potassium (5.5 mEq/L) and causes serious arrhythmia in both adults and children. The mechanisms of hyperkalemia are classified as follows: 1) excessive potassium intake, 2) transcellular movement of intracellular potassium into the extracellular space, and 3) decreased renal excretion of potassium [1]. Infants and young children are prone to dehydration as they have a larger ratio of body surface area to volume with greater losses of insensible transpiration than adults [1]. Dehydration leads to a decreased glomerular filtration rate, which potentially causes a reduction in renal potassium excretion.

Current therapeutic drugs for hyperkalemia that aim to deal with the decreased renal excretion of potassium are loop diuretics, thiazide diuretics, and potassium-binding resins. Loop diuretics and thiazide diuretics increase the urinary-potassium excretion, and potassium-binding resins increase the gastrointestinal excretion of potassium. However, loop diuretics and thiazide diuretics may not be effective in patients with a limited glomerular filtration rate [2], and the effectiveness and safety of potassium-binding resins in children are not clear [3]. Therefore, new drugs that safely and effectively address pediatric hyperkalemia caused by decreased renal excretion of potassium are necessary.

To develop such therapeutics and evaluate their effectiveness, an animal model of pediatric hyperkalemia is required. According to the literature, an adult rat hyperkalemia model has already been developed by the combined administration of amiloride (Ami), a potassium sparing diuretic, and potassium chloride (KCl) [4]. We also reported that administration of Ami together with nafamostat, a known causative drug of hyperkalemia, caused hyperkalemia in male adult rats [5]. We referred to these models and altered them for application in young rats. To determine the model-inducing effects of furosemide (FURO), a loop diuretic and known therapeutic drug for hyperkalemia, serum concentration of potassium and urinary-potassium excretion were examined. As a new therapeutic candidate for hyperkalemia, selective Compound 21 (C21), a nonpeptide angiotensin II type 2 receptor (AT2R) agonist, was studied in our hyperkalemia model [6]. We chose C21 as the inhibition of AT2R along with that of bradykinin type II receptor (BK2R) is known to decrease renal potassium excretion and increase plasma potassium concentration in response to overnight high dietary potassium loading in mice [7]. Thus, we hypothesized that C21 may affect hyperkalemia.

In this study, we developed a young rat hyperkalemia model with decreased renal excretion of potassium and examined the effect of C21 as a new therapeutic candidate in

the model.

Materials and Methods

Animals

Five-week-old male Wistar-Imamichi rats, weighing 100–120 g, were used in this study (Japan SLC Inc. Shizuoka, Japan). The animals were delivered to the laboratory animal center one week before the experiment for acclimatization. The animals were housed under constant humidity and temperature, were kept in a 12 h light/dark cycle, and were maintained on both a certified diet and tap water ad libitum. Animals were fasted for 12 h prior to the experiment.

This study was approved by the Research Center for Laboratory Animals, Dokkyo Medical University (approval number 1374). The study complied with the latest act and guidelines, the Act on Welfare and Management of Animals (Ministry of Environment) and the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan).

Reagents and drugs

Ami was purchased from Sigma-Aldrich (MO, USA) and dissolved in normal saline. FURO was purchased from Nichi-Iko Pharmaceutical Co., Ltd. (Toyama, Japan). C21

104 was purchased from MedChemExpress (NJ, USA) and dissolved in dimethyl sulfoxide
105 (DMSO), polyethylene glycol 300 (PEG 300), polyoxyethylene sorbitan monooleate
106 (Tween 80), and normal saline at a ratio of 2:8:1:9 [8]. DMSO was purchased from
107 FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). PEG 300 and Tween 80
108 were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). A triad of
109 anesthetics was used: medetomidine hydrochloride (1 mg/mL), midazolam (5 mg/mL),
110 and butorphanol tartrate (5 mg/mL) were mixed at a ratio of 4:4:5. Medetomidine
111 hydrochloride was purchased from Nippon Zenyaku Kogyo Co., Ltd. (Fukushima, Japan).
112 Midazolam was purchased from Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan).
113 Butorphanol tartrate was purchased from Meiji Seika Pharma Co., Ltd. (Tokyo, Japan).
114
115 ***Study design for development of a young rat hyperkalemia model***
116 Rats were divided into two groups: KCl + Ami (experimental group) and KCl alone
117 (control group), and the drugs were administered intraperitoneally (i.p.). The
118 experimental group received KCl at doses of 2, 3, 4.5, or 6 mEq/kg and Ami at a dose of
119 3 mg/kg. The control group received KCl at doses of 2, 3, 4.5, or 6 mEq/kg and normal
120 saline in place of Ami. KCl (0.3 mol/L) was administered in two to four divided doses,
121 where each dose was 3 min apart, so that a single dose did not exceed the LD_{50} of KCl in

rats (1.9 mEq/kg) [9]. After all drugs were administered, each rat was placed in a metabolic cage unit (SHINANO Manufacturing Co., Ltd., Tokyo, Japan) for 6 h. After a 6 h-urine collection, rats were anesthetized with a triple anesthetic (0.13 mL/kg, i.p.), and blood was drawn from the inferior vena cava in an open position. Subsequently, euthanasia was performed by exsanguination.

Study design for administration of FURO, a positive control for treating hyperkalemia

The hyperkalemia model was created by the combined administration of KCl (6 mEq/kg, i.p.) and Ami (3 mg/kg, i.p.). FURO was administered orally to rats as a positive control at single or repeated doses. In the single-dose study, rats received FURO (30 mg/kg) 1 h after the administration of KCl and Ami. In the repeated-dose study, rats were administered FURO (30 mg/kg) 1, 3, and 5 h after the administration of KCl and Ami. The doses FURO were determined by referring to the clinical doses and half-life of FURO [10]. Clinical doses were converted to animal doses using the body surface area conversion method [11]. The initial dose of FURO was 10 mg/kg, which is equivalent to the clinical dose in children with hyperkalemia. However, in our preliminary study, a single dose of FURO (10 mg/kg) did not improve hyperkalemia (data not shown). Thus, we studied a single dose and repeated doses of FURO (30 mg/kg). Normal saline was

140 administered to the control rats. The rats were kept in metabolic cages, except at the time
141 of administration. Six hours after the administration of KCl and Ami, urine was collected,
142 and blood was drawn.
143
144 ***Study design for administration of C21, a candidate drug for hyperkalemia***
145 This hyperkalemia model was similar to that in the study of FURO administration.
146 C21 was administered orally to rats as a candidate drug at a single dose of either 10 mg/kg
147 or 30 mg/kg 1 h after the administration of KCl and Ami. C21 doses were determined as
148 the estimated maximum doses that would be tolerated by rats by referring to a previous
149 study [12]. Rats in the control group were administered a C21 solvent (DMSO, PEG 300,
150 Tween 80 and normal saline).
151
152 ***Measurements of urine output and electrolyte concentrations in serum and urine***
153 Urine output was measured as the volume after six-hour urine was collected. Urine
154 and blood samples were centrifuged at 1200 rcf and 20 ℃ for 20 min, and supernatants
155 of urine and serum were collected to measure electrolyte concentration. A portion of the
156 urine sample was diluted eight-fold with distilled water. Potassium and sodium
157 concentrations in the serum and urine samples were measured using a Fuji Dry-Chem

158 System (Fuji Dry-Chem 3500V, Fujifilm Corporation, Tokyo, Japan) with an ion-
159 selective electrode (Fuji Dry-Chem Slide Na-K-Cl, Fujifilm Corporation, Tokyo, Japan).
160 Urinary electrolyte concentrations were corrected using urine creatinine concentrations.
161 Creatinine concentrations in the urine were measured as follows. A portion of the urine
162 sample was diluted two-folds with distilled water. The QuantiChrom™ Creatinine Assay
163 Kit (BioAssay Systems, Hayward, CA, USA) was used to quantify creatinine
164 concentrations (mg/dL). The diluted urine sample（2.5 μL）was mixed with reagent A
165 (25 μL), reagent B (25 μL), and distilled water (50 μL) in a clear-bottom plate. The plate
166 was then gently tapped and mixed. Optical densities were measured immediately and 5
167 min later at 510 nm (Thermo Scientific VarioSkan Flash, Thermo Fisher Scientific Inc.,
168 MA, USA). The optical densities were calculated using a reference curve.
169
170 ***Statistical analyses***
171 We decided the number of rats by referring to the number generally used in
172 experiments involving rats and did not calculate the number needed in advance by power
173 analysis. An *F* test or *H* test was performed on the data of serum concentrations of
174 potassium, urine output, and urinary excretion of electrolytes for comparisons of two
175 groups or three or more groups, respectively. Serum data showed equal variance and were

176 expressed as mean ± standard deviation (SD); most of the urine data showed unequal
177 variance and were expressed as median and 25th and 75th percentiles. A two-sample *t* test
178 was performed on the serum data from studies on the combined administration of KCl
179 and Ami and that of FURO. A One-Way ANOVA was performed on the serum data from
180 the studies of the combined administration of dose-escalation KCl and Ami and that of
181 C21. Two sample *t* test with Welch's correction was performed on the urine data from the
182 studies of the combined administration of KCl and Ami and that of FURO. The Kruskal–
183 Wallis test with Bonferroni correction was performed on the urine data from the studies
184 of the combined administration of dose-escalation KCl and Ami and that of C21. IBM
185 SPSS (version 25; International Business Machines Corp., Armonk, NY, USA) was used
186 for these statistical analyses. The level of significance was set at 5%.
187
188 **Results**
189 ***Development of a young rat hyperkalemia model***
190 The serum-potassium concentration was significantly increased by the combined
191 administration of KCl and Ami compared to the administration of KCl (2 mEq/kg: 5.2 vs
192 4.5 mEq/L, $P = 0.039$, 6 mEq/kg: 6.8 vs 4.1 mEq/L, P=0.005, n=5 each, Fig. 1A).
193 Administration of Ami alone also significantly increased the serum-potassium

194 concentration compared to the control (0 mEq/kg: 4.9 vs 4.4 mEq/L, $P = 0.03$, n=5, 3, Fig.
195 1A). However, the serum-potassium concentration did not exceed 5.5 mEq/L, the upper
196 reference limit, and neither did the urinary-potassium excretion decrease compared to the
197 control at a dose of 2 mEq/kg KCl (Fig. 1B). In contrast, the serum-potassium
198 concentration exceeded the upper reference limit at a dose of 6 mEq/kg KCl, and the
199 urinary-potassium excretion tended to decrease compared to the control (Fig. 1B).
200 Administration of Ami alone significantly decreased the urinary-potassium excretion
201 compared to the control (0 mEq/kg: 0.5 vs 1.0 mEq/mg Cr, P=0.02, n=5, 3, Fig. 1B). As
202 shown in Fig. 2A, the serum-potassium concentration increased in a dose-dependent
203 manner when KCl was administered at increasing doses in the presence of Ami (Fig. 2A).
204 Similarly, a dose-dependent increase in the urinary-potassium excretion was observed
205 (Fig. 2B).
206
207 ***Administration of FURO, a positive control for treating hyperkalemia***
208 FURO was orally administered to rats with hyperkalemia in single or repeated doses
209 of 30 mg/kg. The serum-potassium concentration was significantly decreased by FURO
210 compared to the control (single dose:5.9 vs 6.8 mEq/L, P=0.017, n=7; repeated doses:5.6
211 vs 6.3 mEq/L, P=0.021, n=7, Fig. 3A and 4A). Regarding the urinary-potassium excretion,

212 an increase was shown by repeated doses (1.9 vs 1.4 mEq/mg Cr, *P*=0.03, n=7, Fig. 4B),
213 but no significant difference was observed with a single dose (1.9 vs 2.0 mEq/mg Cr,
214 *P*=0.2, n=7, Fig. 3B). An increase in urine output was observed with a single dose of
215 FURO compared to the control, but at repeated doses, the differences were not significant
216 (*P*=0.059, Table 1). However, the urinary-sodium excretion did not change at either dose
217 (Table 1).
218
219 ***Administration of C21, a candidate drug for hyperkalemia***
220 C21 was orally administered to hyperkalemia rats at single doses of 10 and 30 mg/kg.
221 Two rats died because of aspiration after oral administration of C21 (10 mg/kg and 30
222 mg/kg). The serum-potassium concentration was not changed by C21 at either dose
223 compared with the control (Fig. 5A). The urinary-potassium excretion was not changed
224 by C21 at either dose compared to the control (1.7 vs 1.7 vs 1.4 mEq/mg Cr, *P*=0.14, Fig.
225 5B). There was no significant difference in urine output according to the C21 dose (Table
226 2). There was a difference in the urinary-sodium excretion among the control and both
227 C21 doses. Although the differences in the urinary-sodium excretion were not significant
228 between the control and either C21 dose, there was a tendency of an increase in the
229 urinary-sodium excretion by either C21 dose (*P*=0.054, Table 2).

Discussion

We have developed a young rat hyperkalemia model with reduced the urinary-potassium excretion by changing the original model made by the combined administration of KCl and Ami [4]. In the original model developed by Borok et al., the mean serum potassium increased to 6.73 mEq/L 6 h after the combined administration of KCl (2 mEq/kg) and Ami (3 mg/kg), whereas the mean serum potassium only increased to 5.2 mEq/L at the same dosage of KCl and Ami in the present study. We required a higher dose of KCl to develop a young rat hyperkalemia model, likely due to a difference in renal function at this age; Borok et al. used approximately ten-week-old rats, while we used five-week-old rats. In male Sprague-Dawley rats, the mean serum creatinine concentration in ten-week-old rats was 1.6 times higher than that in six-week-old rats [13]. In another study, the mean glomerular filtration rate in ten-week-old rats was 27% lower than that in six-week-old rats in male F344 rats [14]. Thus, it is conceivable that the renal function for urinary-potassium excretion in five-week-old rats is better than that in ten-week-old rats, necessitating the need for a higher dose of KCl to increase serum-potassium concentration (>5.5 mEq/L) in the present study. Regarding the urinary-potassium excretion, the decrease was not significant after the combined administration

248 of a higher dose of KCl (6 mEq/kg) and Ami compared to the sole administration of Ami
249 (3.3 vs 7.5 mEq/mg Cr, *P*=0.06, n=5, 3, respectively). This may be due to the small
250 number of animals used in this study.

251 The combined use of Ami and KCl resulted in a marked elevation in the serum-
252 potassium concentration, indicating that Ami should be used in combination to develop a
253 rat hyperkalemia model caused by decreased urinary-potassium excretion. Ami is a
254 potassium-sparing diuretic that selectively blocks epithelial sodium channels in the distal
255 nephron (distal convoluted tubule and cortical collecting duct), thereby decreasing
256 potassium excretion by inhibiting sodium–potassium exchange, leading to hyperkalemia
257 [15]. Dose-dependent increases in serum-potassium concentration were observed
258 following the combined administration of ascending doses of KCl and Ami. The doses of
259 4.5 and 6 mEq/kg KCl increased the mean value of the serum-potassium concentration
260 over the upper normal limit (5.5 mEq/L) (Fig. 2A). Therefore, a dose of 6 mEq/kg KCl,
261 in combination with Ami, was chosen to develop a young rat hyperkalemia model.

262 To verify whether this model can be used to evaluate the effect of a candidate drug,
263 we examined the effect of a known treatment against hyperkalemia, FURO, as a positive
264 control. Both dosage regimens decreased the serum-potassium concentration,
265 accompanied by increased urinary-potassium excretion in rats administered repeated

doses. The reason for not observing an increased urinary-potassium excretion with the single dose may be due to large individual differences in the control (Fig. 3B). It is already known that variations in diuretic responses, including urine output and electrolyte excretion, are large in rats [16]. In this previous work, normal saline loading was suggested to reduce the variable results in the study of diuretics [16]. However, the median value of the urinary-potassium excretion in the single dose of FURO was similar to that of repeated doses of FURO (Fig. 3B and 4B). Thus, we assume that a single dose of FURO has the same effect as repeated doses. There was no significant difference with both dosage regimens in urinary-sodium excretion (Table 1). The reason may be the molar doses of NaCl that were administered, as normal saline, as a vehicle in the control group, was about 2.5 times more than that in the FURO group. The difference in the doses was caused by the dilution of the original FURO solution (10 mg/mL) with normal saline by 0.6 times (6 mg/mL); thus, the difference in urinary-sodium excretion was probably not detected among the FURO and control groups in either dosage regimen.

Administration of C21 did not change the serum-potassium concentration at either dose (10 and 30 mg/kg, 6.8 vs 7.2 mEq/L) compared to the control (6.6 mEq/L) (P=0.07), or the urinary-potassium excretion at either dose (10 and 30 mg/kg, 1.7 vs 1.4 mEq/mg Cr) compared to the control (1.7 mEq/mg Cr) (P=0.14, Fig. 5). The C21 solvent was very

viscous. Therefore, we considered the death of the rats to be accidental due to aspiration. We suggest several possible reasons for the ineffectiveness of C21 on the serum-potassium concentration and the urinary-potassium excretion. One could be due to the pharmacokinetic profile, low bioavailability (20-30%), and short half-life (3–6 h) after oral administration of C21 [6]. That is, the concentration of C21 in the blood may not reach an effective concentration. Another reason could be the low protein expression of AT2R in kidneys. One study demonstrated that AT2R protein expression in the kidney was lower in eight-week-old Sprague–Dawley rats [17]. The final reason could be due to a lack of cooperation with a BK2R agonist, as it was shown that the development of hyperkalemia required both the inhibition of AT2R and BK2R in response to overnight high dietary potassium loading in mice [7].

An increase in urinary-sodium excretion was caused by a lower dose of C21. This result was not dose-dependent and was not accompanied by an increase in urine output. Intravenous administration of C21 has been shown to induce natriuresis in rats [12,18]. Therefore, our results may be due to a variation in the pharmacological response and the small number of animals used in this study. Further examination will be needed.

In conclusion, the young rat model of hyperkalemia presented in our work can be used to evaluate the effects of candidate drugs for hyperkalemia in children caused by

decreased urinary-potassium excretion.

Acknowledgments

We thank Mr. Hiromichi Kaneko at Research Center for Laboratory Animals, Dokkyo Medical University School of Medicine, for his support with the animal experiments in this study. We thank Ms. Natsuko Hirabayashi and Mr. Tomonari Handa, medical students assigned to a medical laboratory at Dokkyo Medical University, for their support in this study. We would also like to thank Editage (www.editage.com) for English language editing.

Disclosure statement

The authors have no conflict of interest.

Authors contribution

Y.T., S.T-N., and T.F. designed the study, conducted animal experiments, analyzed the data, and wrote the manuscript. T. Y. and S. Y. reviewed the manuscript.

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372

373 **Tables**

374 Table 1. Changes in urine outputs and urinary-sodium excretion after administration of
375 single-dose furosemide (FURO) (30 mg/kg) and repeated-dose FURO (30 mg/kg) in a
376 young rat hyperkalemia model

			Urine output (ml)	*P* value	Urinary sodium (mEq/mg Cr)	*P* value
single dose	control	Mean	7.0		10.4	
		Median	6.4		5.8	
		25th percentile	6.4		4.3	
		75th percentile	7.0	0.046	14.8	N.S.
	FURO	Mean	8.8		5.5	(0.19)
		Median	8.6		5.1	
		25th percentile	8.3		4.3	
		75th percentile	9.9		6.5	
repeated dose	control	Mean	9.2		6.8	
		Median	9.2		4.3	
		25th percentile	8.6		3.9	
		75th percentile	9.6	N.S.	5.9	N.S.
	FURO	Mean	11.2	(0.059)	6.2	(0.81)
		Median	11.6		5.5	
		25th percentile	10.1		5.4	
		75th percentile	12.4		6.9	

377

378 n=7 each for all groups.
379 Two sample *t* test with Welch's correction was performed.
380 N.S., not significant
381
382

Table 2. Changes in urine outputs and urinary-sodium excretion after administration of Compound 21 (C21) in a young rat hyperkalemia model.

		Urine output (ml)	*P* value	Urinary sodium (mEq/mg Cr)	*P* value
Control	Mean	7.9		5.1	
	Median	7.4		4.9	
	25th percentile	6.7		4.6	
	75th percentile	8.7		5.4	
C21 (10 mg/kg)	Mean	7.3		6.7	
	Median	7.3	N.S. (*P*=0.83)	6.8	0.045
	25th percentile	6.7		6.0	
	75th percentile	7.9		7.4	N.S. (*P*=0.054)
C21 (30 mg/kg)	Mean	7.8		4.5	
	Median	7.7		4.5	
	25th percentile	7.6		4.1	
	75th percentile	7.9		5.0	

n=6, 4, and 4 for control, C21 (10 mg/kg), and C21 (30 mg/kg), respectively
Kruskal-Wallis test with Bonferroni correction was performed.
N.S., not significant

Figures

Fig.1

A **B**

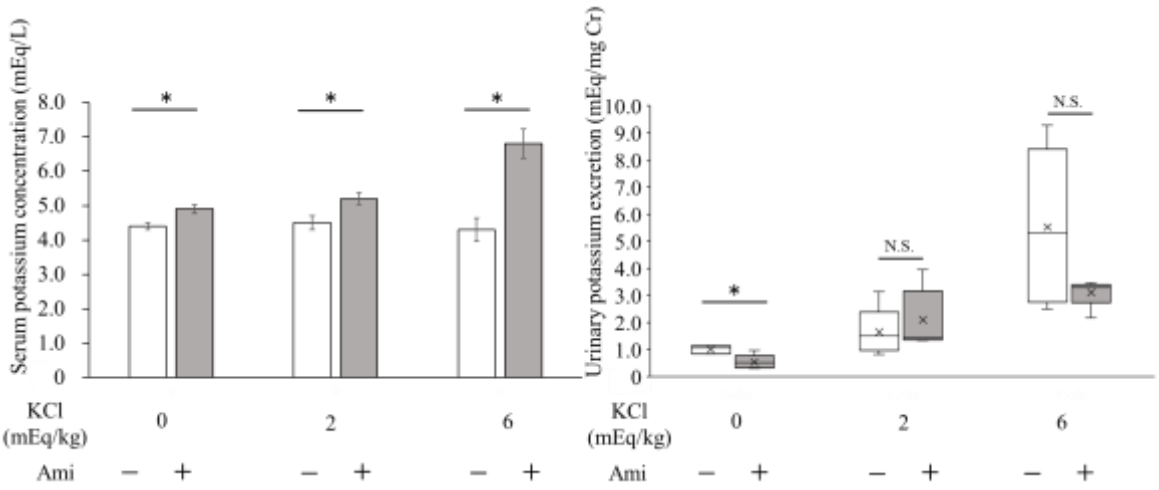
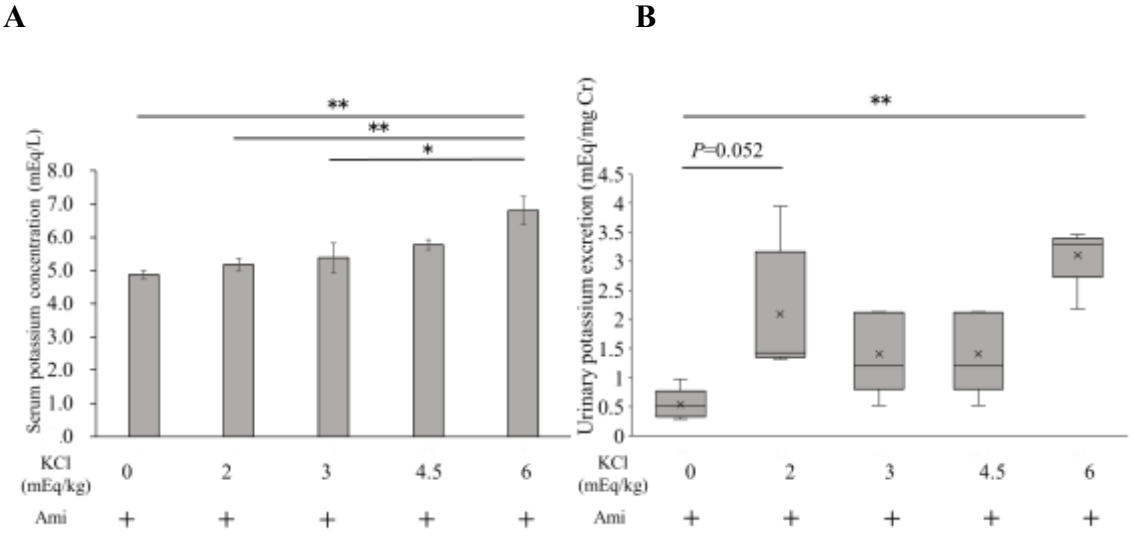
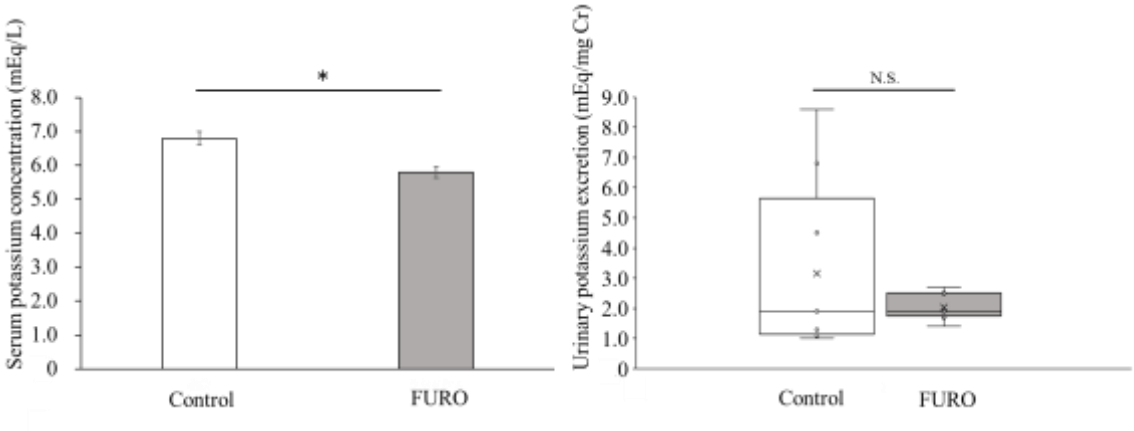


Fig. 2


A
B
**
**
*
Serum potassium concentration (mEq/L)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
.0
KCl (mEq/kg)
0
2
3
4.5
6
Ami
+
+
+
+
+
**
P=0.052
Urinary potassium excretion (mEq/mg Cr)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
KCl (mEq/kg)
0
2
3
4.5
6
Ami
+
+
+
+
+

425 **Fig. 3**

426 **A** **B**



427

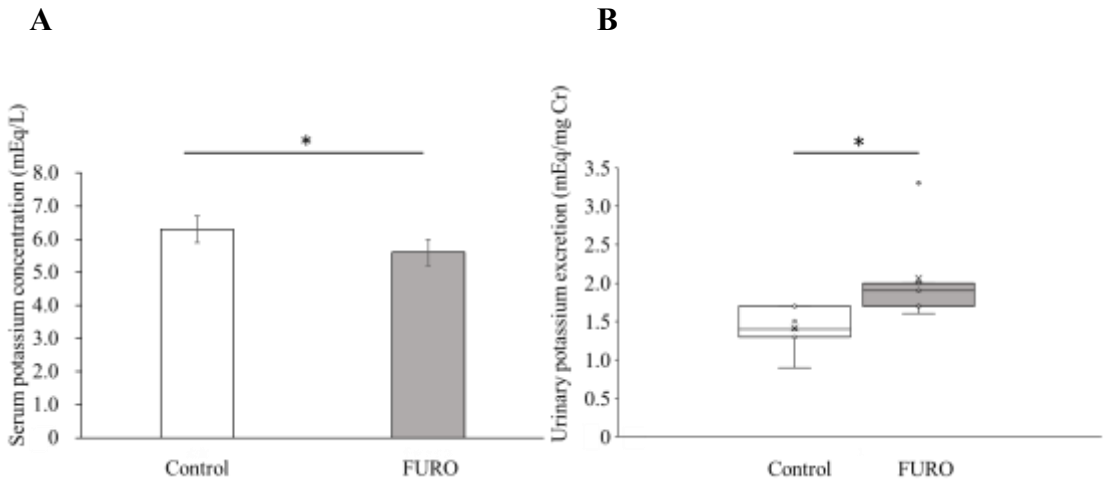
428

429

430

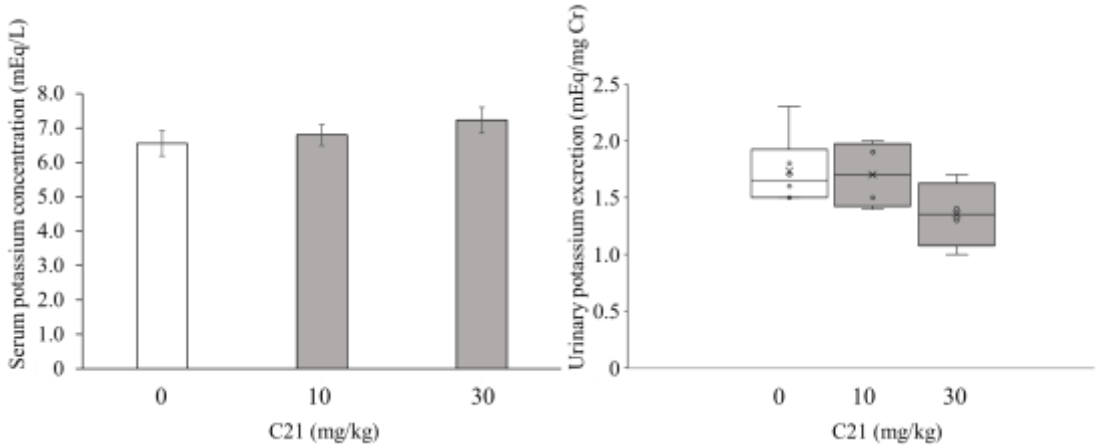
431

Fig. 4


A
B
Serum potassium concentration (mEq/L)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
*
Control
FURO
Urinary potassium excretion (mEq/mg Cr)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
*
Control
FURO

438 **Fig. 5**

439 **A** **B**



440

441

442

443

444 **Figure Legends**

445

446 Fig. 1. Changes in serum-potassium concentration (A) and urinary-potassium excretion
447 (B) after combined administration of KCl and Ami.

448

449 (A) In the control group, KCl (0, 2, and 6 mEq/kg i.p., n=3, 5, and 3, respectively)
450 and normal saline were administered to five-week-old rats (open bar). In the experimental
451 group (n=5 each), KCl (0, 2, and 6 mEq/kg i.p.) and Ami (3 mg/kg i.p.) were administered
452 to five-week-old rats (closed bar). Data are expressed as mean ± SD. A two-sample *t* test
453 was performed. **P*<0.05.

454

455 (B) In the control group, KCl (0, 2, and 6 mEq/kg i.p., n=3, 5, and 3, respectively)
456 and normal saline were administered to rats (open bar). In the experimental group (n=5
457 each), KCl (0, 2, and 6 mEq/kg i.p.) and Ami (3 mg/kg i.p.) were administered to five-
458 week-old rats (closed bar). Data are expressed as box plots. A two-sample *t* test was
459 performed. **P*<0.05.

460 Ami, amiloride; KCl, potassium chloride; SD, standard deviation; N.S., not significant.

461

462 Fig. 2. Changes in serum-potassium concentration (A) and urinary-potassium excretion
463 (B) after combined administration of ascending doses of KCl and Ami.
464
465 (A) Ascending doses of KCl (0, 2, 3, 4.5, and 6 mEq/kg i.p.) and Ami (3 mg/kg i.p.)
466 were administered to five-week-old rats (n=5 each). Normal saline was administered
467 instead of KCl in the KCl group (0 mEq/kg). Data are expressed as mean ± SD. A One-
468 Way ANOVA with Tukey's test was performed. *P<0.05, **P<0.01.
469
470 (B) Ascending doses of KCl (0, 2, 3, 4.5, and 6 mEq/kg i.p.) and Ami (3 mg/kg i.p.)
471 were administered to five-week-old rats (n=5 each). Normal saline was administered
472 instead of KCl in the KCl group (0 mEq/kg). Data are expressed as box plots. The
473 Kruskal-Wallis test with Bonferroni correction was performed. **P<0.01.
474 Abbreviations: Ami, amiloride; ANOVA, analysis of variance; KCl, potassium chloride;
475 SD, standard deviation.
476
477
478 Fig. 3. Changes in serum-potassium concentration (A) and urinary-potassium excretion
479 (B) after single-dose administration of FURO in a young rat hyperkalemia model.

480

481 (A) In the control group (n=7), normal saline was administered to five-week-old rats
482 1 h after combined administration of KCl (6 mEq/kg, i.p.) and Ami (3 mg/kg, i.p.) (open
483 bar). In the experimental group (n=7), single-dose FURO (30 mg/kg, p.o.) was
484 administered to five-week-old rats 1 h after combined administration of KCl (6 mEq/kg,
485 i.p.) and Ami (3 mg/kg, i.p.) (closed bar). Data are expressed as mean ± SD. A two-sample
486 *t* test was performed. **P*<0.05.

487

488 (B) In the control group (n=7), normal saline was administered to five-week-old
489 rats 1 h after combined administration of KCl (6 mEq/kg, i.p.) and Ami (3 mg/kg, i.p.)
490 (open bar). In the experimental group (n=7), single-dose FURO (30 mg/kg, p.o.) was
491 administered to five-week-old rats 1 h after combined administration of KCl (6 mEq/kg,
492 i.p.) and Ami (3 mg/kg, i.p.) (closed bar). Data are expressed as box plots. Two sample *t*
493 test with Welch's correction was performed.
494 Abbreviations: Ami, amiloride; FURO, furosemide; KCl, potassium chloride; N.S., not
495 significant.

496

497

498 Fig. 4. Changes in serum-potassium concentration (A) and urinary-potassium excretion
499 (B) after repeated-dose administration of FURO in a young rat hyperkalemia model.
500
501 (A) In the control group (n=7), repeated doses of normal saline were administered to
502 five-week-old rats at 1, 3, and 5 h after combined administration of KCl (6 mEq/kg, i.p.)
503 and Ami (3 mg/kg, i.p.) (open bar). In the experimental group (n=7), repeated doses of
504 FURO (30 mg/kg, p.o.) were administered to five-week-old rats 1, 3, and 5 h after
505 combined administration of KCl (6 mEq/kg, i.p.) and Ami (3 mg/kg, i.p.) (closed bar).
506 Data are expressed as mean ± SD. A two-sample *t* test was performed. **P*<0.05.
507
508 (B) In the control group (n=7), repeated-dose normal saline was administered to
509 five-week-old rats 1, 3, and 5 h after combined administration of KCl (6 mEq/kg, i.p.)
510 and Ami (3 mg/kg, i.p.) (open bar). In the experimental group (n=7), repeated doses of
511 FURO (30 mg/kg, p.o.) were administered to five-week-old rats 1, 3, and 5 h after
512 combined administration of KCl (6 mEq/kg, i.p.) and Ami (3 mg/kg, i.p.) (closed bar).
513 Data are expressed as box plots. Two sample *t* test with Welch's correction was performed.
514 **P*<0.05.
515 Ami, amiloride; FURO, furosemide; KCl, potassium chloride; SD, standard deviation.

516

517

518 Fig. 5. Changes in serum-potassium concentration (A) and urinary-potassium excretion
519 (B) after administration of C21 in a young rat hyperkalemia model.

520

521 (A) In the control group (n=6), DMSO, PEG300, Tween 80, and normal saline were
522 administered to five-week-old rats 1 h after combined administration of KCl (6 mEq/kg,
523 i.p.) and Ami (3 mg/kg, i.p.) (open bar). In the experimental group, C21 (10 and 30 mg/kg,
524 p.o., n=4 each) was administered to five-week-old rats 1 h after combined administration
525 of KCl (6 mEq/kg, i.p.) and Ami (3 mg/kg, i.p.) (closed bar). Data are expressed as mean
526 ± SD. A One-Way ANOVA was performed.

527

528 (B) In the control group (n=6), DMSO, PEG300, Tween 80, and normal saline were
529 administered to five-week-old rats 1 h after combined administration of KCl (6 mEq/kg,
530 i.p.) and Ami (3 mg/kg, i.p.) (open bar). In the experimental group, C21 (10 and 30 mg/kg,
531 p.o., n=4 each) was administered to five-week-old rats 1 h after combined administration
532 of KCl (6 mEq/kg, i.p.) and Ami (3 mg/kg, i.p.) (closed bar). Data are expressed as box
533 plots. Kruskal-Wallis test was performed.

534 Abbreviations: Ami, amiloride; ANOVA, analysis of variance; C21, Compound 21;
535 DMSO, dimethyl sulfoxide; PEG 300, polyethylene glycol 300; polyoxyethylene sorbitan
536 monooleate, Tween 80; KCl, potassium chloride; SD, standard deviation.
537
538
539