Chronopharmacology of Angiotensin II–receptor Blockers in Stroke-Prone Spontaneously Hypertensive Rats

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Received November 2, 2010; Accepted December 14, 2010

Abstract. Protective effect of valsartan (Val), an angiotensin II (AII)-receptor blocker (ARB), against organ damage is reported to depend on the dosing time in hypertensive patients. Dosing-time–dependent effect of Val on survival of stroke-prone spontaneously hypertensive rats (SHRSP) under a 12-h lighting cycle was examined. Val (4 mg/kg per day) and olmesartan medoxomil (OM) (1 mg/kg per day), another ARB with a slower dissociation from the AII receptor, were given once daily at 2, 8, 14, or 20 HALO (hours after lights on). Dosing-time–dependent differences in plasma drug concentrations and effect on blood pressure (BP) were also evaluated. Survival of SHRSP showed a dosing-time–dependent change during Val therapy, with a peak at 2 HALO and a trough at 14 HALO. OM equally prolonged survival in all groups. The BP-lowering effect persisted for more than 24 h after dosing of Val at 2 HALO and of OM at 2 and 14 HALO, but disappeared at 5.5-h after Val dosing at 14 HALO. Plasma concentrations of Val and OM were higher after dosing at 2 HALO than at 14 HALO. These results suggest that the chronopharmacological phenomenon of Val was partly due to the dosing-time–dependent difference in plasma concentration and subsequent duration of the antihypertensive effect. Slower dissociation of OM from AII receptors might have blunted a potential dosing-time–dependent event.

Keywords: valsartan, olmesartan medoxomil, chronopharmacology, stroke-prone spontaneously hypertensive rats (SHRSP), stroke

Introduction

Angiotensin II (AII)-receptor blockers (ARBs) are widely used for the treatment of hypertension and related diseases. These drugs improve morbidity and mortality in patients with hypertension (1) and are recommended as first-line agents for the treatment of hypertension (2).

There is increasing evidence that the effectiveness and/or toxicity of many drugs can be altered by their dosing-time (3–5). A chronopharmacological approach seems to be desirable for more effective and safe dosage regimens. There are precedents for this approach in clinical practice (6–8).

Valsartan (Val) is an orally active, specific, and selective ARB (9). Daily dosing of the drug in the morning in hypertensive patients caused smooth blood pressure (BP) reduction throughout 24 h, with no significant alterations in BP circadian rhythm (10). In contrast, evening dosing of Val resulted in an improved diurnal/nocturnal BP ratio, especially in patients with the non-dipper pattern, which in turn leads to reduced urinary albumin excretion (11, 12). As the decreased excretion of urinary albumin exerts a renal-protective effect and reduces cardiovascular risk (13), evening Val dosing is thought to be a more effective dosage regimen than morning Val dosing for preventing the occurrence of, and consequent death due to, cerebrovascular and cardiovascular diseases caused by hypertension. This is the first study to address the issue using stroke-prone spontaneously hypertensive rats (SHRSP), an animal model of human stroke (14, 15). To examine a
dosing-time–dependent effect of Val, the drug was given to SHRSP once daily at one of four different time points for 200 days, and the survival of the animals was compared among these trials. In comparison to Val, olmesartan medoxomil (OM), another ARB, dissociates more slowly from AT<sub>1</sub> receptors (16), which led to a hypothesis that the dosing-time–dependent effect of OM is different from that of Val. The chronopharmacological profile of OM was also evaluated in this study.

**Materials and Methods**

**Animals**

Six-week-old male SHRSP/Izm and WKY rats were obtained from Japan SLC Co. (Shizuoka). They were maintained for 3 – 4 weeks before the experiments in two rooms under specific pathogen-free conditions with a controlled light/dark cycle (12:12 h). The lights were switched on and off at 07:00 and 19:00, respectively, in room 1 and at 19:00 and 07:00, respectively, in room 2 (Fig. 1). Two or three rats were kept in one cage. These animals had free access to standard chow and water. The study protocol was approved by the Institutional Review Committee. The experiments were performed in accordance with the Use and Care of Experimental Animals Committee of Jichi Medical University (Tochigi).

**Drugs and dosing**

As the inhibitory action of OM on 125I-AII binding to AT<sub>1</sub> receptor was about four-fold greater than that of Val [IC<sub>50</sub> (concentration required for 50% inhibition): OM, 0.78 nM, Val, 3.1 nM; Ki (inhibition constant): OM, 0.57 nM, Val, 2.2 nM] (17), 4 mg/kg per day of Val and 1 mg/kg per day of OM were selected in this study. The recommended daily dose is generally 80 – 160 mg for Val and 20 – 40 mg for OM in clinical practice. These drugs were suspended in the vehicle (1% tragacanth gum solution) and given to rats once daily by gastric gavage. The doses of Val and OM were adjusted relative to body weight twice a week.

**Experiments**

**Experiment 1. Effects of dosing-time of Val and OM on the survival of SHRSP:** SHRSP were randomly assigned to one of four groups, each with a different time of dosing (2, 8, 14, and 20 h after lights on, HALO; Fig. 1). Each group was further divided into three subgroups (each n = 10) as follows: 1) 4 mg/kg per day Val, 2) 1 mg/kg per day OM, and 3) vehicle. Salt-loading (1% NaCl solution) and dosing of Val, OM, or vehicle was initiated simultaneously to SHRSP at each of the four different times. After treatment, survival was checked daily for 200 days. The survival period of animals that lived for more than 200 days was deemed to have survived for 200 days. Cerebral bleeding was detected in all SHRSP that died during the treatment period.

**Experiment 2. Changes in mean arterial BP in SHRSP during treatment with Val and OM:** SHRSP were randomly assigned to the 2 or 14 HALO group. Each group was further divided into three subgroups as follows: 1) 4 mg/kg per day Val (n = 6), 2) 1 mg/kg per day OM (n = 6), and 3) vehicle (n = 7). Salt-loading was initiated, and Val, OM, or vehicle was given at 2 or 14 HALO once daily for more than 4 weeks. For BP measurements, rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and a polyethylene catheter (PE-50; Becton Dickinson, Franklin Lakes, NJ, USA) filled with heparinized saline (10 U/mL, 0.05 mL/min) was inserted into the left common carotid artery and connected to a pressure transducer (P-3000S; Nidec Copal Electronics Co., Tokyo). The mean arterial pressure (MAP) was recorded continuously with the animals alert, unrestrained, and with free access to food and water. More than 12 h after the surgery, MAP recording was started after dosing of Val, OM, or vehicle at 2 or 14 HALO and was performed for 24 h.

Differences in the area under the mean MAP-time curve between the vehicle and ARB (Val or OM) groups (Area) were calculated using the trapezoid area every 0.5
Lysis Reagent® and total RNA was extracted using QIAzol (50 mg/kg i.p.) and the left ventricle region of the heart was dissected. Tissues were homogenized in QIAzol alone. Salt-loading was initiated, and Val, OM, or vehicle (n = 5). WKY rats in each group (n = 5) had the vehicle (n = 5), 2) 1 mg/kg per day OM (n = 5), and 3) vehicle (n = 5). WKY rats in each group (n = 5) had the vehicle alone. Salt-loading was initiated, and Val, OM, or vehicle was given at 2 or 14 HALO once daily for 4 weeks.

The rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and the left ventricle region of the heart was dissected. Tissues were homogenized in QIAzol Lysis Reagent® and total RNA was extracted using an RNase Mini kit (Qiagen, Valencia, CA, USA), according to the manufacturer’s protocol. Total RNA from each sample was used for preparation of cDNA using the Superscript® VILO™ cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA). Real-time PCR amplification was performed with the SYBR® Premix Ex TaqII (Takara Bio, Otsu) using the Stratagene Mx3005P (Agilent Technologies, La Jolla, CA, USA). The following sequence-specific primers were used: sense, GCT GTG GCA GCT ACC TAT GTG TTG and antisense, AGG TCG TCA TCA TCC CAC GAG for IL-1β (GenBank Accession No., NM_031512); sense, CCA CTT CAC AAG TCG GAG GCT TA and antisense, GTG CAT CAT CGC TGT TCA TAC AAT C for IL-6 (GenBank Accession No., NM_031512); sense, CCA CTT CAC AAG TCG GAG GCT TA and antisense, GTG CAT CAT CGC TGT TCA TAC AAT C for IL-6 (GenBank Accession No., NM_031512); sense, CCA CTT CAC AAG TCG GAG GCT TA and antisense, GTG CAT CAT CGC TGT TCA TAC AAT C for IL-6 (GenBank Accession No., NM_031512); sense, CCA CTT CAC AAG TCG GAG GCT TA and antisense, GTG CAT CAT CGC TGT TCA TAC AAT C for IL-6 (GenBank Accession No., NM_031512); sense, CCA CTT CAC AAG TCG GAG GCT TA and antisense, GTG CAT CAT CGC TGT TCA TAC AAT C for IL-6 (GenBank Accession No., NM_031512); sense, CCA CTT CAC AAG TCG GAG GCT TA and antisense, GTG CAT CAT CGC TGT TCA TAC AAT C for IL-6 (GenBank Accession No., NM_031512); sense, CCA CTT CAC AAG TCG GAG GCT TA and antisense, GTG CAT CAT CGC TGT TCA TAC AAT C for IL-6 (GenBank Accession No., NM_031512); sense, CCA CTT CAC AAG TCG GAG GCT TA and antisense, GTG CAT CAT CGC TGT TCA TAC AAT C for IL-6 (GenBank Accession No., NM_031512). To control a variation in the amount of cDNA available for PCR in different samples, mRNA expression levels of the target sequences were normalized to the expression of an endogenous control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data were analyzed using the comparative threshold cycle method.

Experiment 3. Effects of Val and OM on cardiac interleukin (IL)-1β and IL-6 mRNA expression level: SHRSP and WKY were randomly assigned to the 2 or 14 HALO group. SHRSP in each group was further divided into three subgroups as follows: 1) 4 mg/kg per day Val (n = 5), 2) 1 mg/kg per day OM (n = 5), and 3) vehicle (n = 5). WKY rats in each group (n = 5) had the vehicle alone. Salt-loading was initiated, and Val, OM, or vehicle was given at 2 or 14 HALO once daily for 4 weeks.

The elimination rate constant (ke) was determined by linear regression analysis of the log-linear phase of the plasma concentration–time curve. The elimination half-life was calculated as $t_{1/2} = \ln(2)/ke$.

Method validation

Calibration curves were prepared by adding IS, Val, and OM in methanol to control rat plasma to obtain final concentrations of 1.0 – 100 ng/mL Val and 1.0 – 50 ng/mL OM. Linear regression analysis of the area of the analyte to that of IS versus concentration was used for calibration. Good linearity was obtained [coefficient of determination (r²), ≥0.999]. The detection limits (LOD) of Val and OM, at a signal-to-noise ratio of 3, were 0.22 and 0.46 ng/mL, respectively. The quantification limits (LOQ) of Val and OM, at a signal-to-noise ratio of 10, were 0.73 and 1.53 ng/mL, respectively.

Instrumentation and operating conditions

An Agilent HPLC system was used, and 10 μL samples were injected automatically. A reversed-phase Imtakt Union UK-C18 (50 × 2.0 mm I.D., particle size 3.0 μm) was used. Solvent A (0.1% formic acid in distilled water) and solvent B (0.1% formic acid in methanol) were used as mobile phases for gradient elution (gradient curve: 0 min, 10% B; 0 – 10 min, linear change from 10% to 95% B; 10 – 15 min, 95% B; 15 – 15.01 min, linear change from 95% to 10% B; run time, 20 min). The flow rate was set at 0.5 mL/min. A time-of-flight mass spectrometer (TOF/MS) with an electrospray ionization (ESI) interface (JEOL, Tokyo) was used. Detection was performed by monitoring the positive ions. The theoretical m/z values of [M + H]+ ion were 436.2 for Val, 447.2 for OM, and 515.2 for telmisartan.

Maximum plasma concentration ($C_{\text{max}}$) was calculated from the plasma concentration data of each rat, and the area under the plasma concentration–time curve from 0 to 12 h ($AUC_{0-12}$) was calculated by the trapezoidal rule. The elimination rate constant (ke) was determined by linear regression analysis of the log-linear phase of the plasma concentration–time curve. The elimination half-life was calculated as $t_{1/2} = \ln(2)/ke$. 

Sample preparation

Rat plasma was mixed with 0.5 μL of internal standard solution (IS, 10 μg/mL telmisartan) and 200 μL of methanol. After vortexing and centrifugation (4,000 rpm, 10 min), the supernatant was separated and 5 mL of distilled water was added. The mixture was loaded onto a Waters Oasis HLB SPE column (30 mg) pretreated with 1 mL of methanol, followed by 1 mL of distilled water. SPE columns were washed with 2 mL of distilled water. The analytes were eluted with 2 mL of methanol. The eluate was collected, and then it was evaporated to dryness under a stream of nitrogen at 45°C and reconstituted with 0.5 mL of 50% methanol.

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**Statistical analyses**

Data are shown as means ± S.E.M. Comparison of survival periods was performed by Kaplan-Meier analysis, and other comparisons between the groups were performed by the Bonferroni-Dunn test, following analysis of variance using StatView (SAS Institute, Cary, NC, USA). In all analyses, P values <0.05 were deemed to indicate statistical significance.

**Results**

Experiment 1. Effects of Val and OM dosing-time on the survival of SHRSP (Table 1, Fig. 2)

Survival did not differ significantly among the vehicle-treated subgroups of the four dosing-time groups of SHRSP. Compared with the vehicle, Val significantly prolonged the survival of rats in the 2, 8, and 20, but not 14 HALO trials. The survival period in the 2 HALO group was significantly (P < 0.05) greater than that in the 14 HALO group. OM significantly prolonged the survival of animals in all groups, but no dosing time–dependent change in survival was detected.

Experiment 2. Changes in mean arterial BP in SHRSP during treatment with Val and OM (Figs. 3, 4)

During repeated dosing with Val, MAP decreased significantly in the 2 and 14 HALO trials (P < 0.01 and P < 0.05, respectively). Significant MAP reduction persisted for 24 h in the 2 HALO group and disappeared from 5.5 to 24 h in the 14 HALO group. OM also significantly lowered MAP (P < 0.01), and its BP-lowering effect was observed for 24 h in the 2 and 14 HALO groups. The area calculated by formula (1) was greater in the 2 HALO group than the 14 HALO group (800 mmHg·h vs. 375 mmHg·h, respectively) after Val dosing and was similar between the 2 HALO (645 mmHg·h) and 14 HALO (724 mmHg·h) groups after OM dosing.

**Table 1.** Survival days of SHRSP after the repeated dosing of valsartan (Val) or olmesartan medoxomil (OM) at 2, 8, 14, or 20 HALO

<table>
<thead>
<tr>
<th>Dosing-time</th>
<th>2 HALO</th>
<th>8 HALO</th>
<th>14 HALO</th>
<th>20 HALO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>45.1 ± 3.5</td>
<td>50.2 ± 2.8</td>
<td>43.6 ± 3.8</td>
<td>63.5 ± 15.4</td>
</tr>
<tr>
<td>Val</td>
<td>135.8 ± 21.8**,#</td>
<td>109.6 ± 21.1*</td>
<td>77.6 ± 20.6</td>
<td>136.2 ± 21.4*</td>
</tr>
<tr>
<td>OM</td>
<td>183.2 ± 16.8**</td>
<td>190.0 ± 10.0***</td>
<td>173.7 ± 17.5**</td>
<td>175.1 ± 17.1**</td>
</tr>
</tbody>
</table>

Mean ± S.E.M., n = 10 in each group. *P < 0.05, **P < 0.01 vs. each vehicle; #P < 0.05 vs. 14 HALO.

![Fig. 2. Effects of Val, OM, and vehicle dosing-times on the survival of SHRSP. n = 10 in each group; *P < 0.05 vs. 14 HALO.](image-url)
Experiment 3. Effects of Val and OM on cardiac IL-1β and IL-6 mRNA expression level (Fig. 5)

It was reported that cardiac IL-1β and IL-6 mRNA levels are elevated in SHR rats compared to those in WKY rats (18). To evaluate the anti-inflammatory effects of Val and OM in SHRSP, we measured cardiac mRNA expression levels of IL-1β and IL-6 not only in SHRSP, but in normotensive WKY controls. Cardiac IL-1β and IL-6 mRNA expression levels in the 2 HALO group were significantly higher than those in the 14 HALO group in WKY rats. These parameters significantly \( P < 0.05 \) increased in SHRSP (except for IL-1β mRNA expression in the 2 HALO group), and the time-dependent differences disappeared. Val slightly decreased cardiac IL-1β and IL-6 mRNA expression levels in the 2 HALO group, but not in the 14 HALO group, and subsequently these parameters were significantly lower or tended to be lower in the 2 HALO group than in the 14 HALO group. OM significantly decreased or tended to decrease cardiac IL-1β and IL-6 mRNA expression levels in the 2 and 14 HALO groups, and no time-dependent differences were detected.

Experiment 4. Chronopharmacokinetics of Val and OM (Table 2, Fig. 6)

Plasma concentrations of Val and OM after dosing at 2 HALO were higher than those after dosing at 14 HALO. The \( C_{\text{max}} \) and AUC\(_{0-12} \) of Val in the 2 HALO group tended to be greater than those in the 14 HALO group. These parameters of OM were significantly greater in the 2
HALO group than in the 14 HALO group.

Discussion

SHRSP is an animal model of severe hypertension with subsequent development of stroke (14, 15). By replacing drinking water with a 1% NaCl solution, these animals show accelerated organ damage such as stroke, cerebral edema, renal dysfunction, and cardiac hypertrophy (19, 20). Sodium loading enhances the activity of angiotensin-converting enzyme (ACE) in the brain and aorta in these animals, which in turn, can exaggerate organ damage (21, 22).

Although many chronopharmacological profiles have been reported for antihypertensive agents, limited information is available regarding the dosing-time–dependent prevention of organ damage and improvement of survival rate.

1) Organ damage: the preventive effect of the ACE inhibitor trandolapril against cardiac hypertrophy in hypertensive rats with aortic banding was greater after dosing in the resting period than in the active period (23). On the other hand, the beneficial effect of nitrendipine, a calcium-channel blocker, on cardiac hypertrophy was greater after dosing in the active period in SHR (24).

2) Survival rate: the effects of temocapril, an ACE inhibitor, on the mortality of SHRSP depend on the time of dosing, with the maximum effect observed after dosing in the early resting period (25).

This study yielded the additional observation that the survival of SHRSP depended on the dosing-time of Val, with a peak at 2 HALO (early resting period) and a trough

<table>
<thead>
<tr>
<th>Dosing-time</th>
<th>2 HALO</th>
<th>14 HALO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>137.7 ± 41.2</td>
<td>62.1 ± 15.9</td>
</tr>
<tr>
<td></td>
<td>AUC&lt;sub&gt;(0-12)&lt;/sub&gt; (ng h/mL)</td>
<td>529.5 ± 188.3</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>OM C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>84.2 ± 11.3*</td>
<td>38.0 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>AUC&lt;sub&gt;(0-12)&lt;/sub&gt; (ng h/mL)</td>
<td>382.8 ± 38.0**</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>3.7 ± 0.4</td>
</tr>
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Mean ± S.E.M., n = 3 in each group. *P < 0.05, **P < 0.01 vs. 14 HALO.

Fig. 5. Effects of Val and OM on cardiac IL-1β and IL-6 mRNA expression levels in WKY rats and SHRSP. Mean value of WKY in the 2 HALO group was set to 1.0. White columns, 2 HALO; closed columns, 14 HALO. Means ± S.E.M., n = 5.

Fig. 6. Effects of dosing-time on the pharmacokinetics of Val and OM in SHRSP. Open circles, 2 HALO dosing; closed circles, 14 HALO dosing. Means ± S.E.M., n = 3.
at 14 HALO (early active period).

Hermida et al. reported that in contrast to dosing in the morning, Val dosing at bedtime reduced the percent of non-dipper hypertensive patients (morning dosing: before treatment, 58.8%; after treatment, 57.8%; bedtime dosing: before treatment, 63.2%; after treatment 17.3%) and decreased urinary albumin excretion, a marker of organ damage (morning dosing: before treatment, 29.5 mg/24 h, after treatment, 25.2 mg/24 h; bedtime dosing: before treatment, 29.7 mg/24 h, after treatment 17.5 mg/24 h) (12). Their data suggest that the morbidity and mortality of hypertensive patients on Val therapy at bedtime are improved to a greater extent than those of patients given the drug in the morning, which is supported by the findings of the present animal study.

Several mechanisms have been proposed for drug-induced prolongation of life span in SHRSP: 1) reduction of BP (20, 26, 27), 2) modulation of inflammation (26, 28), 3) prevention of extracellular matrix accumulation (29), 4) inhibition of superoxide production (28), and 5) reduction of platelet aggregation (27). In this study, we examined the dosing-time–dependent effects of Val on BP and IL-1β and IL-6 levels, the markers of inflammation.

In this study, repeated Val dosing significantly decreased BP during 24 h in the 2 HALO group, but this significant decrease disappeared at 5.5 h after dosing of the agent at 14 HALO. Since MAP in WKY rat was about 120 mmHg (30), dosing of Val at 2 HALO showed the great BP-lowering effect. The observation suggests that the longer duration of the BP-lowering effect of Val at 2 HALO is involved in the mechanism of the greater improvement of survival rate in the 2 HALO group. Over the past two decades, several studies have shown that the pharmacokinetic properties of many agents are altered by their dosing-time in animals and humans (31). The chronopharmacological events are considered to involve daily variations in absorption, distribution, metabolism, and excretion of these agents (31). In this study, the plasma concentration of Val tended to be higher after dosing at 2 HALO than after dosing at 14 HALO. Although the Cmax and AUCO–12 of Val were higher after dosing at 2 HALO than after dosing at 14 HALO, the t1/2 of the two trials did not significantly differ. Elimination half-life (t1/2) reflects the ability of metabolism and excretion of an agent from the body. Therefore, it is speculated that the metabolism and excretion of Val were not affected by the dosing-time. In addition, the values of Cmax and AUCO–12 were defined by the balance between absorption and elimination of an agent. Thus, daily variations in the absorption of Val, but not its metabolism or excretion, seem to be involved in the dosing-time–dependent changes in plasma drug concentration. Although plasma concentrations of Val at 6 and 12 h after dosing were not different between the 2 and 14 HALO trials, the BP-lowering effect persisted for 24 h only in the 2 HALO group. These findings suggest that the BP-lowering effect of Val does not depend on the plasma concentration of the parent drug alone. We do not have any definite explanations, but it remains to be determined that active metabolite(s) of Val might contribute to this observation. Further experiments are needed to evaluate this issue.

Elevated endogenous AII is believed to cause organ damage (32). For example, Takai et al. reported that although BP was not lowered, trandolapril reduced tissue ACE activity and prolonged survival in SHRSP (33). AII has significant proinflammatory actions on the vascular wall, including the production of reactive oxygen species, inflammatory cytokines, and adhesion molecules (34). To evaluate another mechanism for the dosing-time–dependent effect of Val, we measured cardiac IL-1β and IL-6 mRNA, the inflammatory cytokines. These parameters in WKY rats were higher during the rest period (2 HALO) than during the active period (14 HALO) in this study. Similar data were reported in the brain of normotensive rats (35, 36). In addition, cardiac IL-1β and IL-6 mRNA levels were shown to increase in SHR (18), which was confirmed in this study. Val has been reported to decrease renal IL-1β mRNA expression in SHRSP (37) and vascular IL-1β and IL-6 mRNA expression in mice with vascular injury (38). In this study, Val tended to decrease cardiac IL-1β and IL-6 mRNA levels in the 2 HALO group, but not in the 14 HALO group. Thus, it is likely that the protective effect of Val against organ damages through its inhibitory action on inflammation was greater after dosing at 2 HALO, which might contribute to the prolonged survival in the 2 HALO group. The chronopharmacokinetic profile of Val seems to also be involved in the dosing-time–dependent effect of Val on inflammatory processes.

In this study, OM significantly prolonged the survival of SHRSP during repeated dosing at 2, 8, 14, and 20 HALO. No dosing-time–dependent difference in the effects of OM was detected. Although the plasma concentration of OM was higher after dosing at 2 HALO than after dosing at 14 HALO, BP decreased similarly more than 24 h after dosing of the agent at 2 or 14 HALO. In this study, 4 mg/kg per day of Val and 1 mg/kg per day of OM, which had equipotent inhibitory actions on AII binding to AT1 receptors (17), were used. However, compared with Val, the dissociation of OM from AT1 receptor was slow [Kd (dissociation rate constant)-OM, 0.0042 min−1 and Val, 0.0099 min−1; t1/2 (half-life)-OM, 166 min, Val, 70 min] (16). Such the characteristics may have contributed to the present findings that although the plasma concentration of OM was higher after dosing at 2
HALO than after dosing at 14 HALO, BP and cardiac inflammatory cytokines similarly decreased in both trials. Thus, it is speculated that the blocking effect of OM on AT1 receptor after dosing at 14 HALO was similar to that after dosing at 2 HALO for 24 h under the present condition. These pharmacological profiles of OM may diminish a potential dosing-time–dependent change in the protective effect against organ damages.

In summary, this study showed that the mortality of SHRSP was improved to a greater extent after dosing of Val in the resting period than in the active period. The dosing-time–dependent change in survival of SHRSP seems to be partly due to the dosing-time–dependent difference in the plasma Val concentration and subsequent duration of the antihypertensive effect and inhibition on inflammatory processes. Slower dissociation of OM from the AII receptor might have blunted such a chronopharmacological phenomenon under the present condition.

Acknowledgments

This study was supported by the Japan Research Foundation for Clinical Pharmacology. This study was subsidized by JKA through its promotion funds from KEIRIN RACE [902006].

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