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Influence of losartan intake on the circadian rhythm of melatonin secretion in humans

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It has been reported that losartan, an angiotensin II receptor blocker, alters the circadian rhythm of melatonin secretion and significantly reduces melatonin production. However, this finding has been confirmed at the animal experiment level only, and there are no reports of studies in humans. Therefore, we performed this study to confirm the reproducibility of the aforementioned findings of animal experiments in humans. Ten male subjects who were in good general health and free from any medical condition were recruited for this study. After a preliminary observation period of 7 days, the subjects received oral losartan treatment, 50 mg daily for 7 days. Blood samplings for measurement of the plasma melatonin concentrations were performed on day 7 of the preliminary observation period and day 7 of the losartan treatment period. The circadian rhythm of melatonin secretion after the 7-day treatment with losartan showed no significant difference from that recorded before the losartan administration. The significant decrease of the home blood pressure was observed on the afternoons. The blood samples showed significant decrease of the serum sodium and uric acid levels, along with a significant increase of the serum potassium level. The pharmacological actions of losartan at the ordinarily used clinical dose level were confirmed in humans, however, no significant inhibitory effect of the drug on melatonin secretion could be confirmed. These results are expected to be useful for guiding the proper use of angiotensin II receptor blockers.

1. Introduction

Angiotensin is known to be closely involved in the regulation of blood pressure (Ferrario et al. 1998; Carey et al. 2000). Baltatu et al. (2002) reported that angiotensin II receptor blockers (ARBs) altered the circadian rhythm of melatonin secretion, and significantly reduced melatonin production. Losartan, an ARB, inhibited pineal melatonin synthesis by 35% ($p = 0.03$) when administered at the dose of 10 mg/kg for 4 days to normal-blood-pressure Wistar-Kyoto rats (WKY). This finding has been confirmed only at the animal experiment level, and there are no reports yet of similar studies in humans.

Melatonin is a hormone that is secreted by the pineal gland of vertebrates, however, its functions have remained unknown for a long time. However, it has been reported that melatonin is related to adjustment of the biological rhythm (Cavallo 1993; Lerchl et al. 1995). Blood levels of melatonin have been shown to peak at night and fall to their nadir in the daytime, indicating the existence of a marked circadian rhythm in melatonin secretion (Penev and Zee 1997). The amount of the hormone released in the night is reported to be 50- to 100-fold that during daytime in humans. Furthermore, the volume of melatonin secretion is also known to decrease immediately upon exposure to strong light at night. The physiological secretory rhythm is controlled by the biological clock in the hypothalamic suprachiasmatic nucleus, however, it is actually synchronized with the light/shade cycle of the outside environment (Lerchl et al. 1995; Reiter 1995; Penev and Zee 1997). Existence of individual differences has also been reported in the circadian rhythm of melatonin secretion. However, it has been suggested that the circadian rhythm remains constant, if the lifestyle remains constant, in any given person (Selmaoui and Touitou 2003; Charles et al. 2009).

In the Japanese guideline for the management of hypertension 2009 (JSH2009), ARBs are positioned as the drug class of first choice for the treatment of metabolic syndrome, from the viewpoint of correction of visceral fat obesity and improvement of the insulin resistance (Ogihara et al. 2009). In recent years, numerous drug combinations containing ARBs with diuretics and/or Ca channel antagonists have been developed and launched in the market, and use of such drug combinations is expected to increase in the future. Metabolic syndrome is also known to be closely associated with the biological clock (Patel 2009; Violanti et al. 2009; Lam and Ip 2010), with suggestions of the existence of a significant relationship with the circadian rhythm of melatonin secretion, however, the findings are limited to those from animal experiments at present (Korkmaz et al. 2009).

From the point of view of the clinical significance of relationships among hypertension, including as a component of metabolic syndrome, biological rhythms and antihypertensive treatment, it is important to confirm the relationship between melatonin and ARBs in humans. Basic information on the relationship between ARBs and the biological rhythm of melatonin

secretion is expected to serve as relevant information for all clinical trials related to treatment for hypertension. Therefore, we conducted this study to confirm, in humans, the ability of losartan, demonstrated in previous animal experiments, to alter the circadian rhythm of melatonin secretion and decrease the synthesis of melatonin.

2. Investigations and results

2.1. Melatonin sampling

All subjects tolerated losartan well and completed the study. The mean melatonin values were low during daytime (less than 10 pg/ml), and increased during the night (38.6 ± 29.03 pg/ml before treatment and 41.8 ± 37.19 pg/ml after the treatment, at 04:00 hours) (Fig. 1a, 1b). The peak levels were 40.2 ± 30.92 pg/ml before treatment, and 43.3 ± 36.34 pg/ml after the treatment. The AUC was 259.7 ± 180.35 pg h/ml before treatment, and 291.7 ± 230.57 pg h/ml after treatment. We found no significant differences in the peak level or AUC after the treatment as compared with the values recorded before treatment (Fig. 1c, 1d).

2.2. Blood pressure

After 7 days of treatment, losartan produced a significant reduction of the mean systolic blood pressure measured at home on the two afternoons prior to the melatonin samplings, from 124.5 ± 10.05 to 116.0 ± 6.86 mmHg ($p = 0.008$) (Fig. 2b), whereas it had no effect on the clinic blood pressure measured at the time of the melatonin sampling or other home blood pressure values (Fig. 2a, 2b).

2.3. Safety assessment

All hematologic and biochemical data were within the normal ranges both before and after treatment. On the other hand, the mean serum levels of sodium, uric acid and insulin were significantly decreased after the treatment, as compared to the values recorded before the treatment (Table). Furthermore, the mean serum level of aspartate aminotransferase (AST) and potassium were significantly increased after the treatment as compared to the values recorded before the treatment (Table). In the lifestyle questionnaire, one subject complained of difficulty in falling asleep after three days of taking losartan. One subject each complained of headache and giddiness after taking losartan for 5 days. One subject each complained of a runny nose, itching of the skin and itching of the eyes after 6 days of losartan treatment.

3. Discussion

In this study, we evaluated the effect of losartan on the biological rhythm of melatonin secretion in ten male subjects who were confirmed to be in good general health by the principal investigator (PI). We compared the AUCs and peak plasma melatonin concentrations after the 7-day losartan treatment period (Losartan 50 mg once a day, after breakfast, for seven days), and examined the inhibitory effect of losartan on melatonin synthesis, reported previously from animal experiments (rats) (Baltatu et al. 2002). No significant alteration of the circadian rhythm of melatonin secretion was observed after the 7-day treatment with losartan, as compared with that recorded prior to this treatment (Fig. 1). The blood levels of melatonin peaked during the night (around 02:00 to 04:00 hours), and reached its nadir during the day (Fig. 1a, 1b). These findings were consistent with previous reports (Touitou et al. 1984; Selmaoui and Touitou 2003). The peak values in the ten subjects of this study varied from less than 10 pg/ml to more than 100 pg/ml. Previous reports about the peak plasma melatonin concentrations in humans also have reported greater than 10-fold differences among individuals (Touitou et al. 1984; Nathan et al. 1996).

Losartan and its active metabolite (EXP3174) are known to cross the blood-brain barrier, as reported from basic animal experiments (Fregly and Rowland 1991; Song et al. 1991; Li et al. 1993; Zhuo et al. 1994; Polidori et al. 1996, 1998; Culman et al. 1999; Wang et al. 2003) and one human experiment conducted to investigate the improvement in cognitive function by the drug (Tedesco et al. 2002). Because human data were generally lacking, we were unable to arrive at a definitive conclusion about the ability of losartan to cross the blood-brain barrier. However, if there was a misclassification (losartan cannot cross the blood-brain barrier), we would have expected it to bias the results toward the null hypothesis before starting this study.

Melatonin represents a precisely timed hormonal message of darkness and is an important output signal of the circadian rhythm; its functional importance is particularly evident in photoperiodic animals, including both the nocturnal rats and diurnal humans, who read the melatonin signal to time the appropriate period for reproduction in accordance with environmental conditions (Redman 1997; Korf et al. 2003).

Based on the association reported between melatonin secretion and sleep in humans (Arendt and Marks 1986; Wurtman and Zhdanova 1995; Mishima et al. 2000; Scheer and Czeisler 2005), we considered that if the animal experiment finding of an inhibitory effect of losartan on melatonin synthesis could be reproduced in humans, it may be possible to explain the insomnia reported as an adverse effect of losartan. However, we could not reproduce the results of the animal experiments in this study. One of the causes for this could be the significantly smaller doses of the drug given to the subjects in this study (50 mg/body) as compared to the doses administered to the rats (10 mg/kg) in the animal experiments. Also, some subjects may have very slight melatonin secretion capacity, and the peak secretion levels of this hormone may differ by about 10-fold among individuals (data not shown). We believe that there were no methodological problems in this study, because the secretion patterns of melatonin before and after the losartan administration were similar in the study subjects (Fig. 1a, 1b). On the other hand, because one subject reported difficulty in falling asleep after three days of losartan administration, the sampling schedule may have posed a problem. We set the losartan administration period in this study at 7 days, based on the results of a Japanese phase I study of losartan (Nakashima et al. 1995). When we planned this schedule, we thought that blood samplings for melatonin each night might pose an ethical problem, because of the potentially high cost to the patient. We think that the salivary sampling method (Kozaki et al. 2011) may be preferable, if the schedule of nightly samplings can be changed. Saliva is particularly useful if repeated sampling is required. In this context, it has been reported that the secretory rhythm of melatonin remains constant in humans, if the lifestyle remains constant (Charles et al. 2009). However, we believe that if the salivary samplings led to a reduction in the quality of sleep, the secretory rhythm of melatonin would be affected. In addition, the salivary sampling method also has some other problems. A melatonin extraction procedure is usually essential, especially since the levels in the saliva are generally about 40% of those in the plasma (de Almeida et al. 2011). The melatonin concentration in cotton saliva collection samples is significantly lower than that in passive saliva collection samples at higher levels of melatonin (Kozaki et al. 2011).

No significant changes of the clinic blood pressure were noted following the losartan treatment (Fig. 2a). However significant

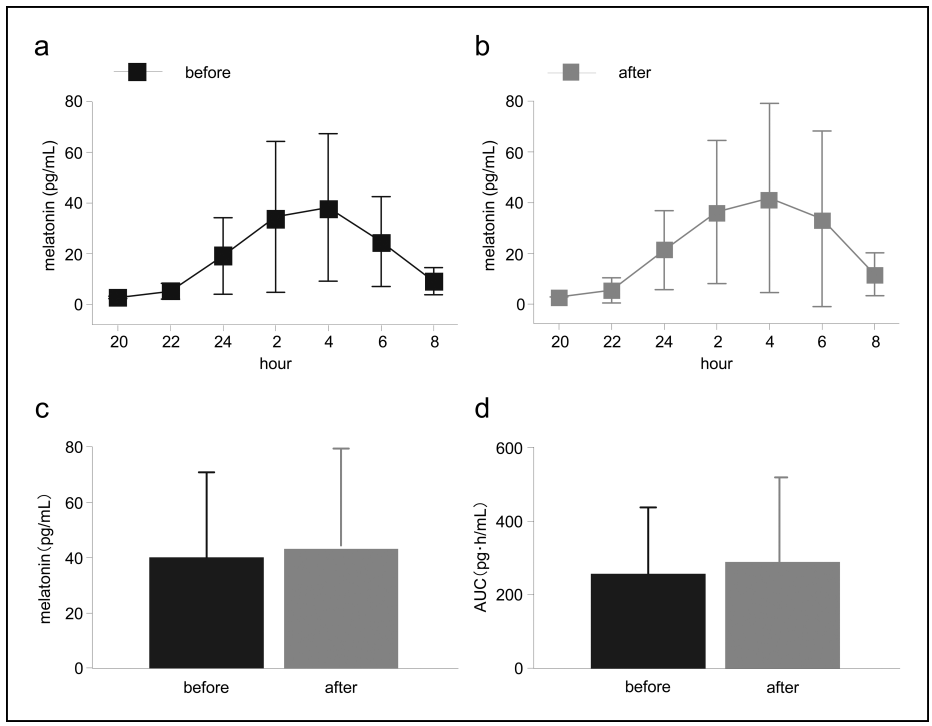

Fig. 1: Plasma melatonin levels in the 10 subjects between 20:00–08:00 hours. (a, b) Hourly mean plasma melatonin levels before treatment (a) and after treatment (b). (c, d) Mean peak melatonin levels (c) and the AUCs (d). Data are mean ± SEM. AUC: area under the curve.

decrease of the home blood pressure from 124.5 ± 10.1 mmHg to 116.0 ± 6.9 mmHg was observed between average of afternoon on day 6–7 of the preliminary observation period and average of afternoon on day 6–7 of the losartan treatment period (Fig. 2b). In the Japanese phase I study of losartan (Nakashima et al. 1995), administration of losartan at the dose of 50 mg daily for one week produced a significant decrease of the morning, afternoon and evening blood pressure values, as compared with the values recorded before the start of losartan treatment. In the post-marketing surveillance study, 5,340 Japanese subjects were included in analysis of efficacy, the effect began to appear by two weeks after the start of losartan administration (Oshima et al. 2003). Furthermore, according to the Japanese mega clinical trial "J-HEALTH", the maximal hypotensive effect of losartan was seen in 3–6 months (Naritomi et al. 2008). It has been reported that the antihypertensive effect of losartan is influenced by the pretreatment blood pressure, and that a marked reduction of the blood pressure is observed in patients with high blood pressure values prior to the start of treatment, and a much slighter decrease in patients with almost normal blood pressure prior to the treatment (Naritomi et al. 2008). Because this clinical study was targeted on healthy adults, it was not surprising that the effect of losartan in reducing the blood pressure was only slight in this study.

In regard to its pharmacological actions, losartan and its active metabolite (EXP3174) inhibit the AT1 receptor specifically and in a sustained manner, resulting in inhibition of constriction of the vasucular smooth muscle and aldosterone secretion from the adrenal cortex, and thereby, decrease of the blood pressure (Sadoshima 2002). Aldosterone promotes water and the salt reabsorption, and potassium excretion (McIntyre et al. 1997). As other special effects, losartan is known to promote uric acid excretion and have an inhibitory effect on platelet aggregation (Levy et al. 2000; Hamada et al. 2008). According to a Japanese study, losartan promotes uric acid excretion by inhibiting urate transporter 1 (URAT-1) in the proximal renal tubules (Hamada et al. 2008). Other ARBs do not have this effect of decreasing the serum uric acid level, and losartan is recommended for the treat-

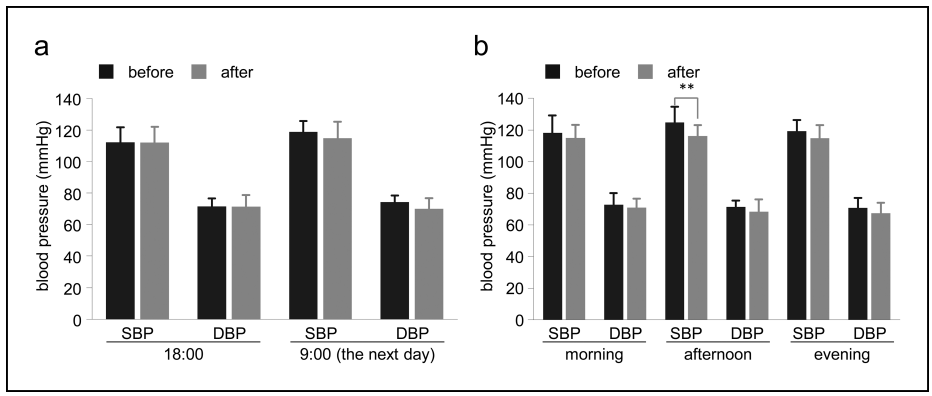

Fig. 2: Blood pressure at the time of the melatonin samplings (a) and at home (b). (a) Blood pressure measured at the time of the melatonin sampling by the medical staff at 18:00 (before) and 09:00 (after) hours. (b) Blood pressure at home was measured by the subjects themselves, once in the morning, once in the afternoon and once in the evening during both the preliminary observation period (7 days) and the losartan treatment period (7 days). Data are mean ± SEM of the values on day 6–7 of the preliminary observation period (before) and day 6–7 of the losartan treatment period (after) $^{**}p < 0.01$ was performed by the paired t-test to determine the statistical significance of any differences in the values between before and after losartan treatment. SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table: Analysis of the hematological and biochemical parameters following losartan treatment

item	before	after	p value	item	before	after	p value
Hematology				**Biochemistry**			
WBC (1,000/mL)	6.0 ± 1.99	5.7 ± 1.38	0.558	Total protein (g/dL)	7.1 ± 0.29	6.9 ± 0.33	0.041*
RBC (10,000/mL)	472.9 ± 26.92	477.8 ± 28.00	0.11	Creatinine (mg/dL)	0.8 ± 0.09	0.8 ± 0.06	0.231
Hemoglobin (g/dL)	14.9 ± 1.06	15.0 ± 1.12	0.262	eGFR	87.8 ± 9.83	90.3 ± 6.62	0.323
Hematocrit (%)	44.1 ± 2.13	44.3 ± 2.44	0.407	Na (mEq/L)	140.7 ± 1.42	137.2 ± 1.14	< 0.001***
				K (mEq/L)	4.2 ± 0.25	5.0 ± 0.40	< 0.001***
Biochemistry				Uric acid (mg/dL)	6.5 ± 1.19	5.6 ± 0.74	0.007**
Platelet (10,000/mL)	26.7 ± 6.36	27.4 ± 7.47	0.337	CK (U/L)	92.6 ± 37.35	93.9 ± 26.27	0.867
ALT (U/L)	16.2 ± 5.88	19.2 ± 9.76	0.074	hsCRP (ng/mL)	10628.3	5429.7	0.301
AST (U/L)	15.7 ± 3.50	18.7 ± 5.68	0.010*		± 25831.38	± 12902.11	
γ-GTP (U/L)	30.7 ± 19.39	33.3 ± 23.53	0.207	FBS (mg/dL)	95.1 ± 8.39	91.1 ± 6.33	0.066
Total bilirubin (mg/dL)	0.8 ± 0.42	0.8 ± 0.46	0.555	Insulin (mmU/mL)	4.4 ± 1.74	3.2 ± 0.81	0.019*

WBC: white blood cell, RBC: red blood cell, ALT: alanine aminotransferase, AST: aspartate aminotransferase, γ-GTP: γ-glutamyl transpeptidase, eGFR: estimated glomerular filtration rate, Na: sodium, K: potassium, CK: creatine kinase, hsCRP: high-sensitivity C-reactive protein, FBS: fasting blood sugar Paired t-test was performed to distinguish statistical difference between before and after treatments.

ment of hypertension in patients with gout and hyperuricaemia in JSH2009. Furthermore, losartan has also been shown in other clinical trials to exert an inhibitory effect of platelet aggregation by competing for the thromboxane A2 receptor (Levy et al. 2000).

On the other hand, blood biochemistry revealed a significant decrease by 3.5 mEq/L of the serum sodium, significant increase of by 0.8 mEq/L of the serum potassium, and a significant decrease by 0.9 mg/dL of the serum uric acid level (Table). It is thought that these changes are caused by the inhibitory effect of losartan on aldosterone secretion on the adrenal cortex (Gandhi et al. 1996) and uricosuric action on the renal proximal tubules (Hamada et al. 2008), which are recognized pharmacological actions of losartan. Despite these effects, no clear hypotensive effect of losartan was observed.

While the study findings endorsed the pharmacological actions of losartan at the dose usually used in clinical settings, the inhibitory effect of the drug on melatonin secretion shown in animal experiments could not be confirmed. Some possible reasons for this were thought to be the difference in the dose of the drug between the rat experiments and this study in humans, and the schedule of the blood samplings for melatonin. These

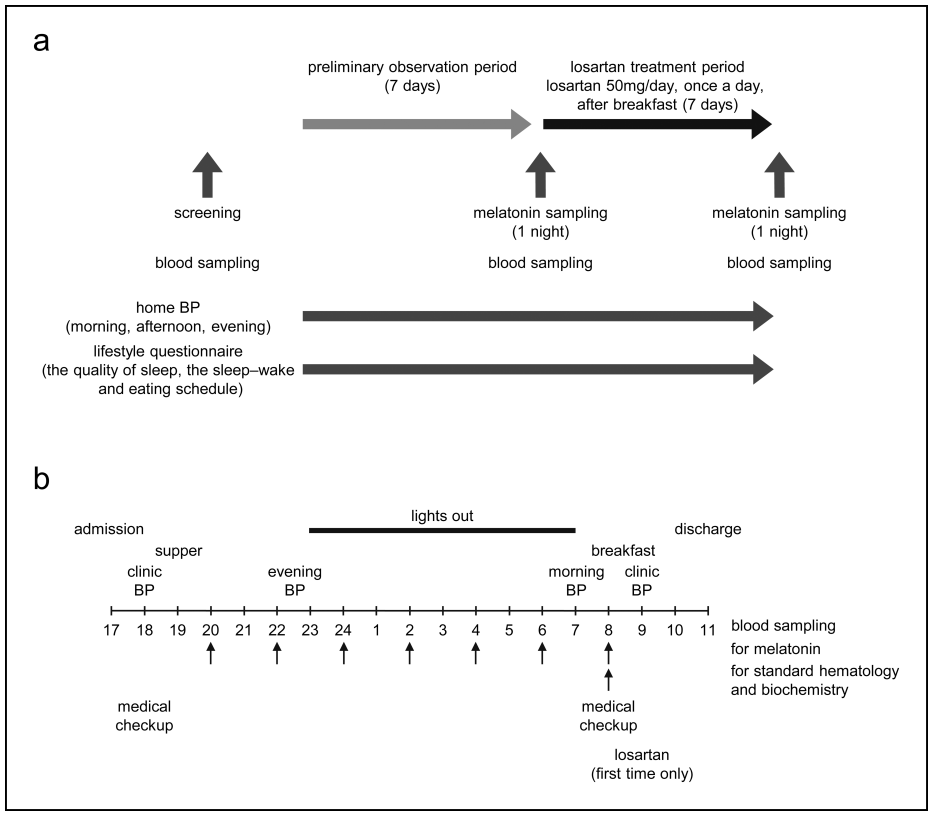

Fig. 3: Study design (a). After the screening and preliminary observation period of 7 days, the subjects received oral losartan at 50 mg per day for 7 days. On day 7 of the preliminary observation period and day 7 of the losartan treatment period, blood samplings were performed for measurement of the plasma melatonin concentrations. The subjects were asked to measure their blood pressure at home and maintain a lifestyle questionnaire about the quality of sleep and the sleep-wake / eating schedule during the 14-day study period. Melatonin sampling (b). On day 7 of the preliminary observation period and day 7 of the losartan treatment period, the subjects provided blood samples for melatonin measurement every 2 hours from 20:00 to 08:00 hours and for standard hematologic and biochemical measurements at 08:00 hours. "Lights out" was implemented for 8 hours, from 23:00 to 07:00 hours. Blood pressure was measured by the medical staff at 18:00 and 09:00 hours. In the morning and evening hours, the subjects measured their blood pressure by themselves. After the blood sampling schedule, the subjects received another medical examination. The subjects took a 50 mg losartan tablet in the morning after breakfast (only on day 1 of the losartan treatment period).

results are, nonetheless, expected to be useful in guiding proper use of ARBs. However, the relationship between ARB and the biological rhythm of melatonin secretion needs to be studied in the future, because the possible melatonin-secreting inhibitory effect of losartan in humans cannot be entirely negated by the results of only this clinical study alone.

4. Experimental

4.1. Subjects

Ten male subjects, aged between 24 and 44 years old (mean ± standard deviation (SD), 34.9 ± 6.9), with a mean body height of 174.1 ± 3.58 cm, mean body weight of 67.7 ± 7.85 kg, and mean body mass index (BMI) of 22.3 ± 2.19 kg/m^2 were recruited for this study. All subjects were in good general health and free from any medical condition at the time of the experiment. None had a history of hypertension or sleep disorders. They were instructed to abstain from alcohol and antihypertensive supplements during the 14-day study period. During this term, they were asked to maintain a regular sleep-wake schedule (go to bed between 21:00 and 24:00 hours and wake up between 06:00 and 08:00 hours) and regular eating schedule (have breakfast between 07:00 and 09:00 hours, lunch between 11:00 and 14:00 hours, and supper between 18:00 and 21:00 hours).

4.2. Study design

The experiments were carried out in May/June 2011. The subjects were screened according to the blood pressure levels and general health condition. After a preliminary observation period of 7 days, the subjects were administered losartan (MSD Co. Ltd, Tokyo, Japan), at the dose of 50 mg once a day, after breakfast, for 7 days. On day 7 of the preliminary observation period and day 7 of the losartan treatment period, blood samplings were performed for measurements of the plasma melatonin concentrations. During the study period, except for the blood samplings, the subjects went about their daily lives as usual (Fig. 3a).

The subjects were asked to measure their blood pressure at home once every morning, afternoon and evening during the 14-day study period (Fig. 3a). In the morning, the blood pressure was measured within 1 hour of awakening, after micturition, a few minutes after resting in the sitting position, before intake of the drug (during the losartan treatment period) and before breakfast, in the afternoon, the measurements were made within 1 hour prior to lunch, and in the evening, the measurements were made just before the subjects went to bed. Blood pressure was measured using a semi-automatic sphygmomanometer, based on the cuff-oscillometric principle HEM-7080IC (Omron Healthcare Co. Ltd., Kyoto, Japan).

The subjects were asked to fill in a lifestyle questionnaire about the quality of sleep and the sleep-wake/eating schedule.

4.3. Melatonin sampling

The subjects spent day 7 of the preliminary observation period and day 7 of the losartan treatment period, in a Phase One Unit of the Clinical Pharmacology Research Institute of the Tokyo Heart Center. The subjects arrived at the unit before 17:00 hours. After undergoing a medical checkup and taking supper at 19:00 hours, the subjects provided blood samples for plasma melatonin measurements every 2 hours from 20:00 to 08:00 hours (in pitch-dark conditions from 24:00 to 06:00 hours) and for standard hematologic and biochemical examinations at 08:00 hours. "Lights out" was implemented for 8 hours, from 23:00 to 07:00 hours (Fig. 1b).

Blood pressure was measured by the medical staff at 18:00 and 09:00 hours after the subjects had rested in the sitting position for a few minutes, with TM-2250-02 (A&D Co. Ltd., Tokyo, Japan), a semi-automatic digitized device, based on the oscillometric method. The subjects themselves measured the blood pressure, once each in the morning, afternoon and evening. After the blood samplings, the subjects took breakfast, and underwent a medical examination. The subjects took a 50 mg losartan tablet in the morning after breakfast (only day 1 of the losartan treatment period), and left the unit at 11:00 hours.

4.4. Data analysis

The melatonin measurements in the blood samples were performed by SRL Inc. (Tokyo, Japan). Other standard hematologic and blood biochemical examinations were performed by Sanritsu Inc. (Chiba, Japan). Safety was assessed by the frequency and severity of adverse events that were possibly related to the study drug, and changes in the laboratory parameters (standard hematology and biochemistry) from the baseline. Data were expressed as means (± SD). Significance testing was performed using the paired t-test and one-way analysis of variance (ANOVA), followed by Tukey's test. Statistical analyses were performed using IBM SPSS statistics, version 19.0 (IBM Corp., Armonk, NY, USA). Differences with $p < 0.05$ were considered to indicate statistical significance.

4.5. Ethics

The experimental procedures and the procedure for obtaining informed consent were approved by Nihon University, School of Pharmacy, Ethics Research Committee/Tokyo Heart Center Institutional Review Board, and the study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Before participation, every subject was given a description about the procedure and general purpose of the experiment, and provided written informed consent. This study was started after it was registered with the University hospital Medical Information Network - Clinical Trials Registry (UMIN-CTR).

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