2. Methods

2.1. Animals

Aged (7 months old) male SHRSP (SHRSP/Izm, Funabashi-Noen, Chiba Japan) were used in the study ($n=40$). Morphologic and patho-physiological studies showed that this strain was suitable as a model of osteoporosis (Yamori et al., 1991; Fukuda et al., 1992, 1995; Tsuruoka et al., 2001). To avoid the occurrence of stroke during the study, standard rat chow (CE-2 containing 1.18% Ca and 2.5 IU/g vit D3, Japan Clea, Tokyo, Japan) and deionized water with 0.5 % potassium chloride were used from 3 months of age (Sugimoto et al., 1992; Tobian, 1986; Tsuruoka et al., 2001). At 6 months old, the rats were divided into four groups ($n=10$ in each) without any significant differences in body weight among the groups.

The animals were kept in two specific-pathogen-free rooms with a 12-h light–dark cycle and diverse lighting schedules (Yamauchi et al., 1998; Tsuruoka et al., 2000, 2001, 2002; Nishiki et al., 2003). In room 1, lights were on at 0700 h and off at 1900 h at a local time. In room 2, lights were on at 1900 h and off at 0700 h. The temperature and humidity in the rooms were maintained automatically. Two groups were kept in room 1 while two other groups were kept in room 2 until the end of the study. It is reported that most physiological parameters, such as neuronal, humoral, motor, and behavioral functions, are completely re-synchronized within 2 weeks after changing lighting schedules (Turek, 1985; Mrosovsky and Salmon, 1987; Takahashi and Zatz, 1982; Takamure et al., 1991) and this maneuver is well accepted in the fields of chronobiology and chronopharmacology. The following experiments were conducted in accordance with Jichi Medical School Guide for Laboratory Animals.

2.2. Experimental design

2.2.1. Single dosing study

On the experimental day, chow was removed and the animals were placed in other cages to measure body weight about 1 h prior to the experiment. 22-Oxacalcitriol (or maxacalcitriol, 12.5 μg/kg, Generous gift from Chugai Pharmaceutical, Tokyo, Japan) or vehicle was injected in a tail vein at 2 h after lights on (2HALO) and 14 h after lights on (14HALO) (i.e. 0900 h at a local time).

Group 1: 2HALO with 22-oxacalcitriol, n = 10
Group 2: 2HALO with vehicle alone, n = 10
Group 3: 14HALO with 22-oxacalcitriol, n = 10 and
Group 4: 14HALO with vehicle alone, n = 10

The dose, which increased serum Ca concentration, was selected on the basis of our preliminary study. Venous blood samples (0.8 ml) were taken from tail vein before and at 2, 4, 6, 8, 12, 18 and 24 h after dosing. Serum samples were frozen and kept at −80 °C until the assay. These protocols were performed after a 2-week acclimatization period in a cross-over fashion.

2.2.2. Repeated dosing study

The 5 μg/kg of 22-oxacalcitriol or vehicle was injected in a tail vein at 2HALO and 14HALO (i.e. 0900 h at a local time) three times a week for 12 weeks. Body weight was measured two times a week until the end of the study. Blood samples (2 ml) were obtained from tail vein at 0900 h (24 h after the last dose of the drug) just before and at 4, 8 and 12 weeks after the initiation of administration. Four-hour urine specimens were collected just before and at 4, 8 and 12 weeks after the start of the study. For the collection of urine, deionized water (3% of body weight) was given by gastric gavage at 30 min after dosing of 22-oxacalcitriol or vehicle and the animals were separately placed in metabolic cages for 4 h (Tsuruoka et al., 2001). Urine collection was performed 1 day before blood sampling. Both serum and urine were stored at −80 °C until the assay. Both femurs were obtained at the end of the study and frozen at −80 °C.

2.3. Assays

Serum and urine Ca was measured by the orthocresolphthalein complexone method (Connerty and Briggs, 1966) and the ammonium molybdate method (Drewes, 1972) with an auto-analyzer, respectively. Creatinine concentration was measured by the modified Jaffe's reaction with an auto-analyzer. Serum albumin was measured by an enzyme-linked immunosorbent assay kit (Panatest; Wako, Osaka, Japan, (Sugimoto et al., 2002)). Serum 22-oxacalcitriol concentration was measured by liquid chromatography–mass spectrometry (Ishigai et al., 1998). Detection limit was 10 pg/ml. Serum parathyroid hormone (PTH) concentration was measured by an immunoradiometric assay (rat PTH IRMA kit, Immutopics, San Clemente, CA, USA). Its normal range is 10–40 pg/ml (Tsuruoka et al., 2001, 2002). Urine deoxypyridinoline, an index of bone resorption, was measured by reverse-phase high-performance liquid chromatography (Seyedin et al., 1993) and its excretion is expressed as a ratio to creatinine concentration.

Bone density of femurs was determined by dual-energy X-ray absorption (DEXA, DCS-600A, Aloka, Japan). The scan was performed every 2 mm along the axis of the bone from the proximal end. Usually 14–17 scans were made for each bone. An average of the first three proximal scans, four scans of middle part, and last three scans are termed "proximal", "medial", and "distal", respectively. The average of all scans is termed "whole". "Medial" is exclusively cortical bone and "distal" is rich in cancellous bone (Shen et al., 1995; Tsuruoka et al., 2001, 2002).

2.4. Statistics

All data are presented as the means ± S.E. Statistical analysis was performed by analysis of variance or Student's *t*-test as appropriate. Scheffe's *F* test was used as a post-hoc test. *P* values less than 0.05 was regarded as significant.

3. Results

3.1. Single dosing study

Fig. 1 shows the changes in serum Ca concentration during the two different dosing schedules. When 22-oxacalcitriol was injected at 2HALO, serum Ca concentration increased for 6 h and then went down. There was no significant difference between the 22-oxacalcitriol and vehicle trials at 18 h after dosing. When the drug was injected at 14HALO, serum Ca concentration did not significantly increase. In the vehicle groups, serum Ca concentration showed a diurnal change, which is compatible with previous

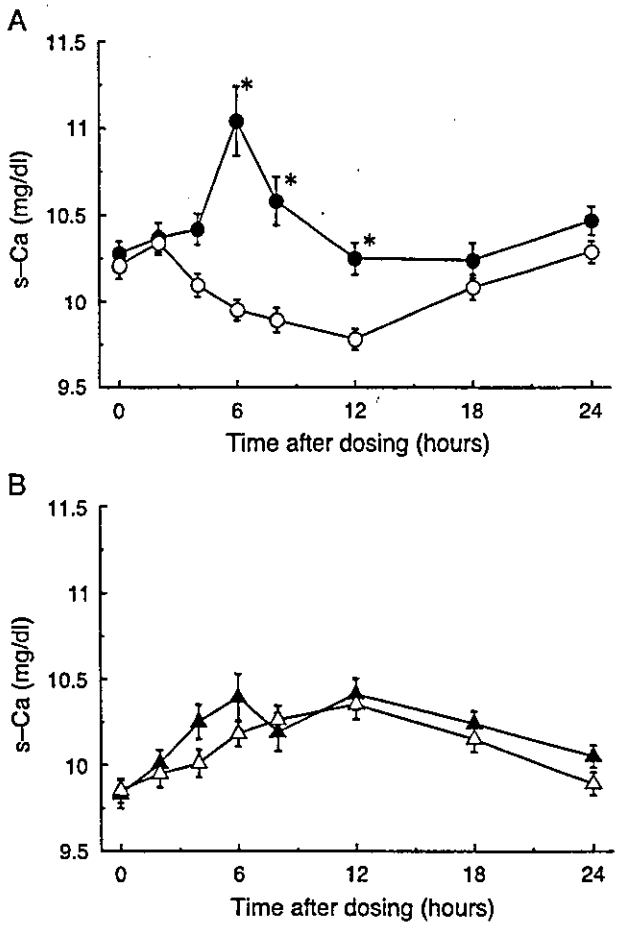

Fig. 1. Serum Ca concentration (s-Ca) after a single dosing of 22-oxacalcitriol or vehicle at 2HALO (A) and 14HALO (B) in aged SHRSP. Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

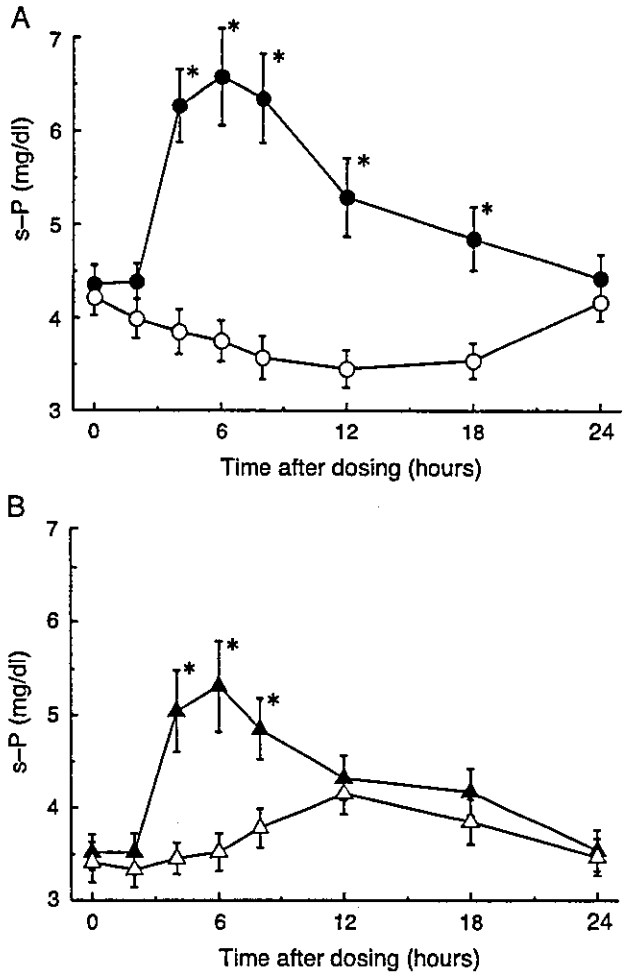

Fig. 2. Serum P concentration (s-P) after a single dosing of oxacalcitriol or vehicle at 2HALO (A) and 14HALO (B) in aged SHRSP. Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

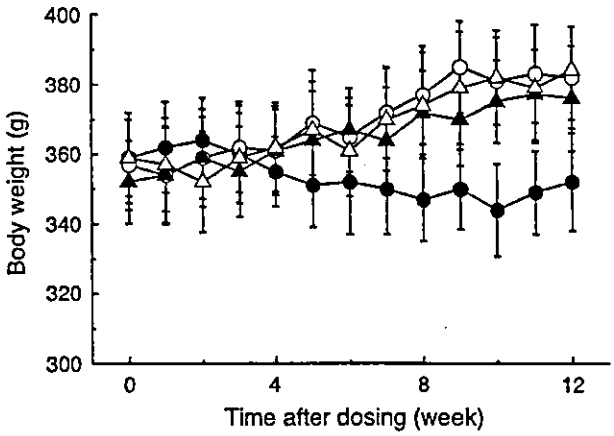

Fig. 3. Body weight during a repeated dosing of oxacalcitriol or vehicle at 2 and 14HALO in aged SHRSP. Mean ± S.E., $n=10$ in each. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

observations (Tsuruoka et al., 2000, 2001, 2002; Nishiki et al., 2003). The difference in the area under the concentration-curve between 22-oxacalcitriol and vehicle trials was significantly ($P<0.01$) greater in the 2HALO trial (2HALO; 8.8 ± 0.9 mg h/dl, 14HALO; 2.1 ± 0.2 mg h/dl). Because ionized Ca concentration is affected by serum albumin concentration, this parameter was simultaneously measured. We found that serum albumin was not influenced by the injection of 22-oxacalcitriol (data not shown). The area under the concentration-curve of serum albumin was 78.8 ± 2.9 and 80.6 ± 2.5 mg h/dl (22-oxacalcitriol and vehicle, respectively) in the 2HALO trial and 74.1 ± 3.2 and 72.5 ± 2.7 mg h/dl (22-oxacalcitriol and vehicle, respectively) in the 14HALO trial. This finding indicates that the serum total as well as ionized Ca concentration actually increased by the drug. We also confirmed the diurnal change in serum total albumin concentration in the vehicle group, which was reported previously (Tsuruoka et al., 2000).

Fig. 2 shows the changes in serum P concentration during the two different dosing schedules. When 22-oxa-

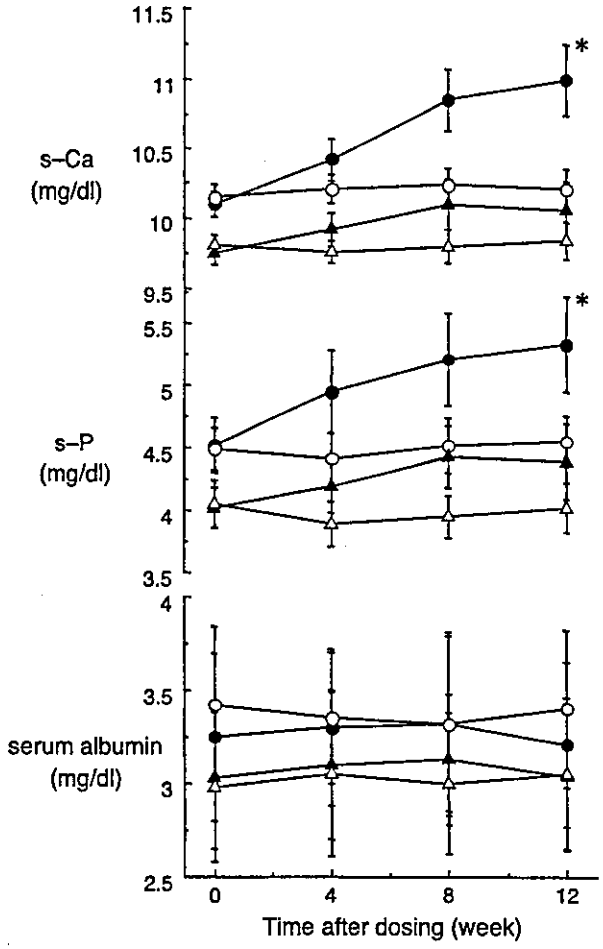

Fig. 4. Serum concentrations of Ca, P and albumin during a repeated dosing of oxacalcitriol in aged SHRSP. Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

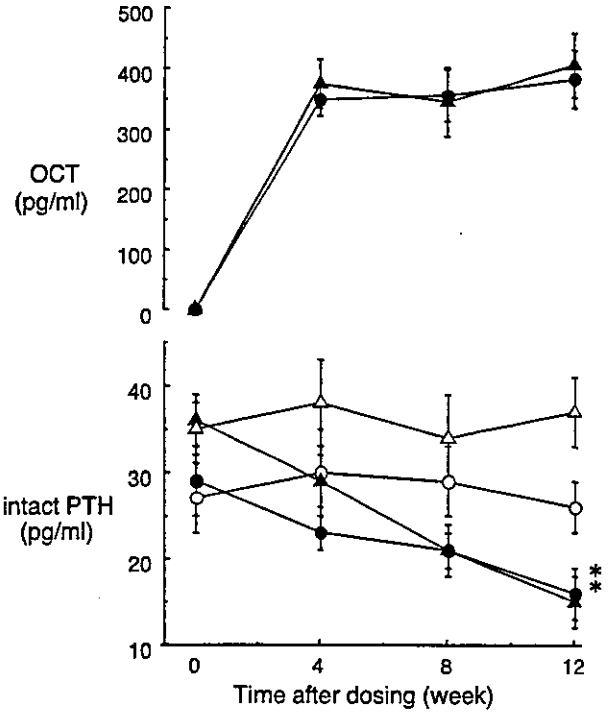

Fig. 5. Serum concentrations of oxacalcitriol and PTH during a repeated dosing of oxacalcitriol in aged SHRSP. Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

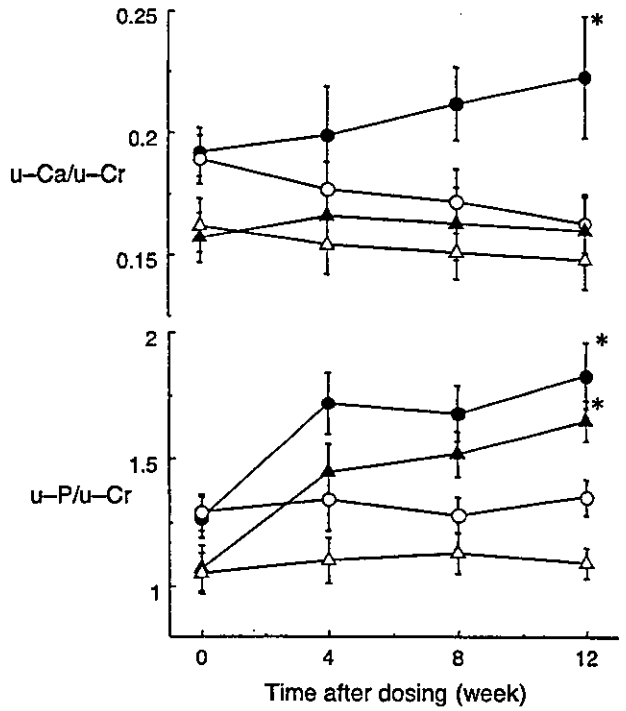

Fig. 6. The ratio of urinary Ca to creatinine (u-Ca/u-Cr) and urinary P to creatinine (u-P/u-Cr) during a repeated dosing of oxacalcitriol in aged SHRSP. Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ oxacalcitriol (14HALO), △ vehicle (14HALO).

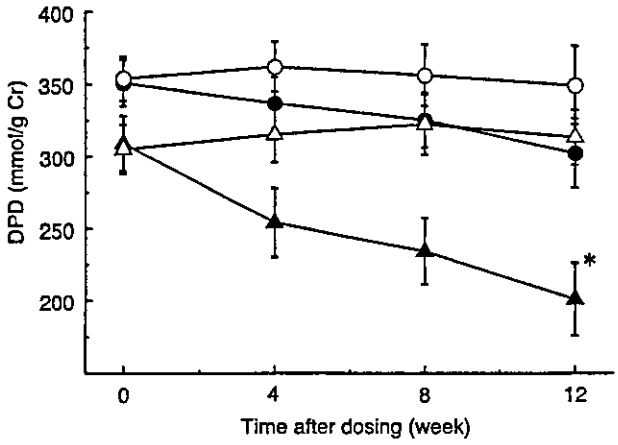

Fig. 7. Urinary deoxypyridinoline excretion during a repeated dosing of oxacalcitriol in aged SHRSP. Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

calcitriol was injected at 2HALO, serum P concentration rapidly increased for 6 h and then went down. There was no significant difference between the 22-oxacalcitriol and vehicle trials at 24 h after dosing. When the drug was injected at 14HALO, serum P concentration increased and was significantly higher than that of vehicle study up to 8 h. In the vehicle groups, serum P concentration showed a diurnal change, which is compatible with previous observations (Tsuruoka et al., 2000, 2001, 2002). The difference in the area under the concentration-curve between 22-oxacalcitriol and vehicle trials was significantly ($P<0.01$) greater in the 2HALO (37.6 ± 3.8 mg h/dl) than in the 14HALO (13.4 ± 1.7 mg h/dl) trials.

3.2. Repeated dosing study

3.2.1. Change in body weight

The change in body weight is shown in Fig. 3. All animals completed the study without any obvious symptoms of stroke. There was a small but significant ($P<0.05$ vs. pre in each) increase in body weight during the study with vehicle at 2HALO and 14HALO, and 22-oxacalcitriol at 14HALO. However, body weight with 22-oxacalcitriol at 2HALO did not increase (mean change: −9 ± 8 g/3 months ($n=10$)).

3.2.2. Serum concentrations of inorganic Ca, P, albumin, albumin, PTH and 22-oxacalcitriol

Serum inorganic Ca and P concentrations before the study were significantly different between the 2HALO and 14HALO trials (Fig. 4). These are compatible with our previous data (Tsuruoka et al., 2000, 2001, 2002) and data in the literature for humans and rats (Shinoda and Seto, 1985; Calvo et al., 1991). Treatment with 22-oxacalcitriol significantly increased serum Ca concentration in the 2HALO but not in the 14HALO trials (Fig. 4). Mean change in serum Ca concentration at the end of the study was 0.8 ± 0.2 mg/dl in the 2HALO trial and 0.3 ± 0.2 mg/dl in the 14HALO trial ($P<0.05$). Similar findings were obtained for serum P concentration (Fig. 4). The mean change in serum P concentration at the end of the study was 0.6 ± 0.2 mg/dl in the 2HALO and 0.3 ± 0.2 mg/dl in the 14HALO trial. Serum albumin concentration, which was measured using same specimens, was not changed by 22-oxacalcitriol in both lighting schedules(Fig. 4). We also evaluated the trough concentration of serum 22-oxacalcitriol, which did not significantly differ between the groups (Fig. 5). Serum PTH concentration before the study was slightly but not significantly higher ($P=0.08$) in the 14HALO than in the 2HALO trial (2HALO; 28 ± 4 pg/ml, 14HALO; 36 ± 3 pg/ml). These levels were within the normal range. Although 22-oxacalcitriol significantly reduced PTH concentration in both trials, the decrease was significantly ($P<0.05$) greater in the 14HALO trial (Fig. 5). Mean decrease in PTH concentration after the treatment was 9.8 ± 2.8 pg/ml in the 2HALO and 16.2 ± 3.2 pg/ml in the 14HALO trial.

3.2.3. Urinary excretions of Ca, P, creatinine and deoxypyridinoline

The urinary ratio of Ca to creatinine (u-Ca/u-Cr) is shown in Fig. 6. The basal value was higher in the 2HALO than in the 14HALO trial, which was consistent with our previous results (Tsuruoka et al., 2001). The drug increased

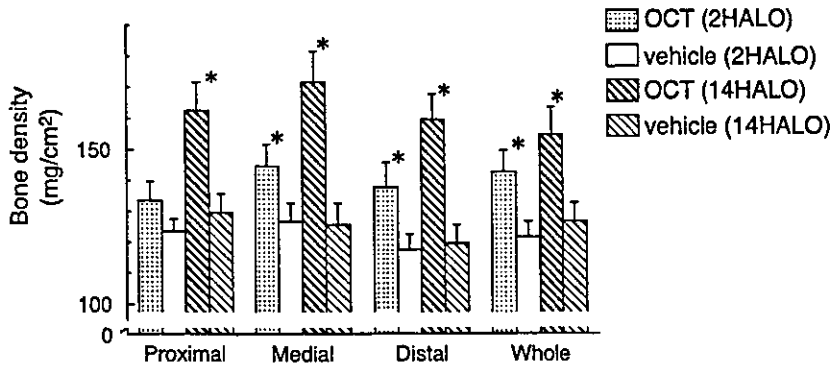

Fig. 8. Bone density of femur at the end of the study, Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. vehicle, #$P<0.05$ vs. morning.

the ratio in the 2HALO but not in the 14HALO trial. The amount of Ca in the 4-h urine showed a similar tendency, which was also comparable with our previous study (data not shown) (Tsuruoka et al., 2001).

The u-P/u-Cr at basal condition was greater in the 2HALO than in the 14HALO trial and was increased by the drug (Fig. 6). However, there was no significant difference in the changes of the ratio by the drug between the 2HALO and 14HALO trials ($P=0.07$). Urinary excretion of deoxypyridinoline was also evaluated. Although this parameter was slightly higher in the 2HALO trial at baseline, the difference was not statistically significant. 22-Oxacalcitriol reduced its excretion in both trials and this change was more prominent in the 14HALO trial (Fig. 7).

3.2.4. Bone density

Bone density of the femur was determined by DEXA at the end of the treatment with 22-oxacalcitriol for 3 months (Fig. 8). Bone density increased during the repeated dosing with 22-oxacalcitriol in both 2HALO and 14HALO trials. However, the increase in the 14HALO group was significantly greater than that in the 2HALO group.

4. Discussion

We have previously reported that the dosing of 1-α(OH) vitamin D3, a prodrug of calcitriol, at 14HALO more reduced the drug-related adverse reactions (hypercalcemia and hyperphosphatemia) and more increased bone density than its dosing at 2HALO in aged SHRSP, a model of osteoporosis (Tsuruoka et al., 2001). 22-Oxacalcitriol was developed to avoid the drug-related hypercalcemia that is commonly observed for calcitriol (Brown et al., 1993; Kubrusly et al., 1993; Farach-Carson et al., 1993). Recently the drug is used for the treatment of secondary hyperparathyroidism with renal osteodystrophy and osteoporosis in clinical situation. In this study, we found that the adverse reactions of 22-oxacalcitriol, such as hypercalcemia and hyperphosphatemia, also differed with its dosing time. We also found that the efficacy of the treatment was greater when drug was given at 14HALO. Thus, 22-oxacalcitriol as well as other analogues of vitamin D have adequate dosing time. In addition, this study showed that the chronopharmacological effects of 22-oxacalcitriol were different from those of other analogues of active vitamin D in several aspects.

One of the most important findings in this study was that the hypercalcemic effect of 22-oxacalcitriol in the 14HALO trial was relatively small. The difference between 22-oxacalcitriol and vehicle in the area under the concentration-curve for serum Ca in the 14HALO trial was almost 1/4 of that observed in the 2HALO trial in our single dosing study (2HALO; 8.8 ± 0.9 mg h/dl, 14HALO; 2.1 ± 0.2 mg h/dl). Our previous single dosing study with 1,25(OH)2 vitamin D3 showed such the difference in the 14HALO trial was about 1/2 of that observed in the 2HALO trial (2HALO; 8.0 ± 1.0. 14HALO; 4.5 ± 0.9 mg h/dl) (Tsuruoka et al., 2000). Based on these findings, we think that the chronopharmacological effect of hypercalcemia was more prominent for 22-oxacalcitriol compared to other analogues of vitamin D3. In the repeated dosing study, serum Ca concentration did not elevate at 14HALO but 2HALO trial. In this study, urinary ratio of Ca to creatinine was not elevated by 22-oxacalcitriol at 14HALO, but increased at 2HALO, which were similar to changes of serum Ca concentration in both trials. Such the chonopharmacological phenomenon was not observed in previous study using 1,25(OH)2 vitamin D3 in identical animal model and 1-α(OH) vitamin D3 in an animal model of osteoporosis (Tsuruoka et al., 2001). Because u-Ca/u-Cr was also higher in 2HALO than 14HALO in this study, the higher serum Ca concentration in the 2HALO trial cannot be explained by the time-dependent difference in the drug sensitivity to renal Ca handling. Moreover, the reduction of deoxypyridinoline excretion and increase of bone density were rather prominent at 14HALO dosing. Therefore, although precise mechanism was not certain, a dosing-time-dependent prevention of Ca release from bone seems to be the reason of this phenomenon. On the other hand, the dosing-time dependent change in the hyperphosphatemic effect of 22-oxacalcitriol was not so prominent as that of serum Ca. Urinary phosphate excretion showed similar tendency to the change of serum phosphate concentration. This finding supports previous observation that 22-oxacalcitriol exerts similar hyperphosphatemic effect to other vitamin D3 analogues but less hypercalcemic effect (Brown et al., 1993; Kubrusly et al., 1993; Farach-Carson et al., 1993).

Another important finding is that the efficacies of 22-oxacalcitriol (prevention of the increase of and loss of bone density) were greater in the 14HALO group. In our previous study using 1,25(OH)2 vitamin D3 in identical animal model, PTH reduction did not differ between the two dosing schedules (Tsuruoka et al., 2002). The mechanism of such a difference between 22-oxacalcitriol and other vitamin D is not obvious at present time. Serum concentrations of trough 22-oxacalcitriol and albumin did not significantly differ between the 2 and 14HALO groups. Therefore, although albumin-unbound 22-oxacalcitriol, an active form(Kobayashi et al., 1994), was not determined in this study, we think that serum concentration of active 22-oxacalcitriol of the two trials did not differ significantly. Thus, pharmacokinetics-related mechanism might not be involved in this phenomenon, which is similar to our previous study using 1-α(OH) vitamin D3 (Tsuruoka et al., 2001). As same as the dosing-time dependent difference of serum Ca concentration, a dosing-time dependent sensitivity to 22-oxacalcitriol of bone seems to be the reason of this phenomenon.

It is generally accepted that rats tends to be active at night (dark phase), while humans do the opposite. However, it is also accepted that serum concentrations of Ca and phosphate show similar fluctuations in the two species (i.e.

higher in light phase and lower in dark phase). When we apply the present findings to the treatment of patients with osteoporosis, we need to consider these differences. It is well-known that serum concentrations of Ca and P are altered by intestinal absorption, which we did not evaluate in this study. On the other hand, some reported genetically altered Ca handling in this strain (Fukuda et al., 1995), which might affect to the results. Future studies need to estimate the contribution to the chronopharmacological effects of the drug.

In conclusion, 22-oxacalcitriol exerts chronopharmacological effect in SHRSP. Adverse reactions (hypercalcemia and hyperphosphatemia) were mild and efficacies (increase of bone density and reduction of PTH) were prominent when 22-oxacalcitriol was given at 14HALO. In addition, the degree of hypercalcemia at 14HALO was slight compared to other vitamin D3 analogues. Although precise mechanisms are not clear, this difference seems to be caused by the change of drug sensitivity in bone cells. These informations will be important to treatment of osteoporosis with less adverse reactions.

Acknowledgements

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Subclinical alteration of taste sensitivity induced by candesartan in healthy subjects

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Aims
There have been case reports of taste disturbance for the angiotensin II receptor blockers losartan and valsartan, but not for candesartan. This study was undertaken to examine whether candesartan causes taste disturbance.

Methods
Candesartan cilexetil (4 mg day^{-1}) or vehicle was given to healthy volunteers ($n = 8$) for 7 days in a randomized, double-blind, placebo-controlled, cross-over design with a 2-week washout period. Clinical gustometry using the filter-paper disc test and electrogustometry were sequentially performed before and at the end of each trial. Serum and salivary zinc concentrations were also measured.

Results
Detection thresholds of four basic tastes (sweet, salty, sour and bitter) determined by the paper disc test were significantly ($P < 0.05$ in all tests) worsened (i.e. score of test increased) after repeated dosing of the drug, although the subjects did not notice such changes. The mean ± SEM (and 95% CI) scores of the four tastes at just before the seventh dosing of candesartan or vehicle was 3.38 ± 0.32 (3.02, 3.74) and 2.63 ± 0.18 (2.18, 3.08) for sweetness, 3.63 ± 0.38 (4.49, 2.77) and 2.63 ± 0.26 (3.27, 1.98) for salt, 4.01 ± 0.42 (3.04, 4.98) and 2.61 ± 0.32 (3.35, 1.87) for sourness, 4.01 ± 0.38 (3.22, 4.80) and 2.99 ± 0.33 (2.24, 3.74) for bitterness, for candesartan and placebo, respectively. Electrogustometry confirmed the candesartan-related taste disturbance. Serum and salivary zinc concentrations were not influenced by candesartan.

Conclusions
These data suggest that candesartan subclinically reduces taste sensitivity after repeated dosing in healthy subjects. Because similar events are reported for losartan and valsartan in case reports, this adverse effect might be a class effect of angiotensin-II receptor blockers (ARBs).

Introduction

Taste disturbance is caused by several endogenous and exogenous factors, about 20% of which are drug-related [1–3]. Antihypertensive drugs have been identified as potential causes of taste disturbance [2, 3]. Fifty percent of patients with taste disturbance have low serum zinc concentrations and some antihypertensive drugs complex with or chelate zinc. Zinc metalloprotein is present in salivary fluid and is important in the maintenance of taste cells.

Captopril, an ACE inhibitor, induces taste disturbance. The captopril molecule contains a thiol-group (–SH) and has been shown to form chelates with zinc [2–4]. However, other ACE inhibitors without the thiol-radical have been reported to cause taste disturbance. The ACE enzyme is a zinc-dependent enzyme. Because

ACE inhibitors, even without thiol groups, need zinc for reaction, the inhibition of ACE by the drug may affect the zinc of the ACE protein in the salivary gland cells, and subsequently may alter taste.

Angiotensin-II receptor blockers (ARBs) are relatively new but widely used antihypertensive agents [5]. ARBs inhibit angiotensin II-mediated effects and induce haemodynamic changes similar to ACE inhibitors. Moreover, taste disturbance is also reported with the ARBs losartan and valsartan [6–9]. To our knowledge, there have been no case reports on candesartan cilexetil (candesartan), another ARB. Therefore, it is interesting to examine whether taste disturbance is a class effect of ARBs. To address this issue, candesartan or placebo was given orally for 7 days in a randomized, double-blind, cross-over design to healthy subjects. Recognition thresholds for four basic tastes (sweet, salty, sour and bitter) by filter paper disc test and detection threshold for electrogustometry were determined at the end of each period.

Methods

Study design

Eight healthy men (29–46 years) were enrolled in this study. All subjects gave written informed consent. They were non-smokers and did not receive any medications throughout the study. Before the initiation of the study, no otolaryngeal disorders were detected in these subjects. The study was of a randomized, double-blind, placebo-controlled, cross-over design with a 2-week washout period. On day 1 (observation period), the detection thresholds for tastes were determined and salivary fluid was obtained at 09.00 h, 12.00 h, 15.00 h and 21.00 h. Subjects took candesartan cilexetil (Blopress®, Takeda Pharmaceutical Co. Ltd, Tokyo, Japan, 4 mg, of powder plus lactose, wrapped in a wafer) or placebo (100 mg of lactose, wrapped in a wafer) at 09.00 h for 7 days from day 2 to day 8. On day 8, an evaluation test and samplings of salivary fluid and blood were performed at 09.00 h, 12.00 h, 15.00 h and 21.00 h for the measurement of zinc and candesartan concentrations. On the day of the taste evaluations, subjects took their breakfast before 07.00 h. For lunch, a similar light meal (sandwich with milk) was served to all subjects just after the evaluation at 15.00 h. Subjects were prohibited from taking any other food or drink (except distilled water) until the end of the study at 21.00 h. After the washout period, an identical protocol was repeated in a cross-over fashion (days 23–30). The protocol was approved by the Ethics Committee of Jichi Medical School.

Samplings of saliva and blood

Spontaneously salivated salivary fluid was collected into a special polyethylene tube after gargling with distilled water three times. The plastic tubes were incubated with 1 M HCl for 30 min and washed with distilled water before use to remove any metals. Blood samples were taken from the cubital vein and serum was transferred into the special tubes after centrifugation. Both salivary fluid and serum were stored at –80 °C until assayed.

Assay

Total zinc concentration was measured by atomic absorption spectrophotometry [10]. Salivary and serum concentrations of candesartan and M-II, an active metabolite, were measured by high-performance liquid chromatography [11]. Detection limits for candesartan and M-II were 1 and 2 ng ml^{-1}, respectively.

Evaluation for taste disturbance

Two types of gustometries were performed in each subject.

1 Semi-quantitative clinical gustometry using a filter-paper disc Semi-quantitative clinical gustometry using filter-paper discs (the filter-paper disc method; Taste disc®, Sanwa Chemical Laboratory, Nagoya, Japan) were performed. This semiquantitative method is routinely used for the evaluation of dysgeusia in the clinical setting [12–14]. In brief, recognition thresholds for four basic tastes (sweet, salty, sour and bitter) were evaluated using the same chemical solutions (sucrose, NaCl, tartaric acid and quinine, respectively). The solutions were sequentially diluted with distilled water into five grades. Concentration number 1 is the lowest and 5 is the highest (0.3, 2.5, 10, 20 and 80% for sucrose, 0.3, 1.25, 5, 10 and 20% for NaCl, 0.02, 0.2, 2, 4 and 8%, for tartaric acid, 0.001, 0.02, 0.1, 0.5 and 4% for quinine). Subjects were asked to gargle with distilled water several times just before each test. A small droplet of each solution was added to filter paper (8 mm diameter), which was placed on the left side of the tongue 2 cm from the tip (i.e. locus for left chorda tympani nerve) of the subjects for 1 s. The test was started from concentration number 1 and gradually increased. Subjects selected a single answer from the following list; sweet, salty, sour, bitter, unidentifiable taste, or no taste. Thresholds for basic tastes were determined by their answer. The order of testing for the four basic tastes was randomly chosen. The test was performed by the same person (ST) throughout the study. Mean thresholds for normal volunteers were less than 3 [13, 15]. We have confirmed that the mean changes among three continuous exami-

nations were -0.17 ± 0.05, -0.22 ± 0.07, -0.14 ± 0.05 and -0.19 ± 0.05, for sweet, salty, sour and bitter, respectively, in healthy subjects ($n = 8$). Thus, the reproducibility of the test was acceptable.

2 Electrogustrometer The electrogustometry routinely used for the evaluation of hypogeusia in the oto-rhino-laryngology clinic was performed according to the method of Tomita *et al.* [12, 13, 15] using commercially available equipment (TR-06®, Rion Co., Ltd, Tokyo, Japan). In brief, a single type stimulation rod was placed on the left side of the tongue 2 cm from the tip (i.e. locus for left chorda tympani nerve) and the electrical stimuli was pulsed from the lowest power (−8 dB) and gradually increased. The smallest stimulus that the subjects noticed was regarded as the detection threshold. Normal range was less than + 14 dB [12, 13, 15]. The test was performed following the filter disc test after gargling with distilled water. The test was performed by the same person (ST) throughout the study. We have previously confirmed that the mean change among three continuous examinations was $+0.8 \pm 0.2$ dB in healthy subjects ($n = 8$). Thus, the reproducibility of the test is acceptable.

Statistics

All data are expressed as the mean ± SEM. Statistical analysis was performed by analysis of variance. Fisher's Protected Least Significance Difference (PLSD) test was used as a *posthoc* test. These analyses were performed using StatView 5 for Windows (SAS Institute Inc, NC). $P < 0.05$ was regarded as significant.

Results

All subjects completed the protocol without any complaints of taste disturbance. Mean blood pressure 24 h before final dosing of the drug was not different between candesartan and placebo (108.5 ± 6.5 and 112.5 ± 4.2 mmHg, respectively, $P = 0.08$). Figure 1a-d shows the recognition thresholds for tastes using filter-paper disc. During the observation periods (day 1 and day 23), the thresholds of four tastes were within the normal range in all subjects and did not differ significantly between day 1 and day 23. The detection thresh-

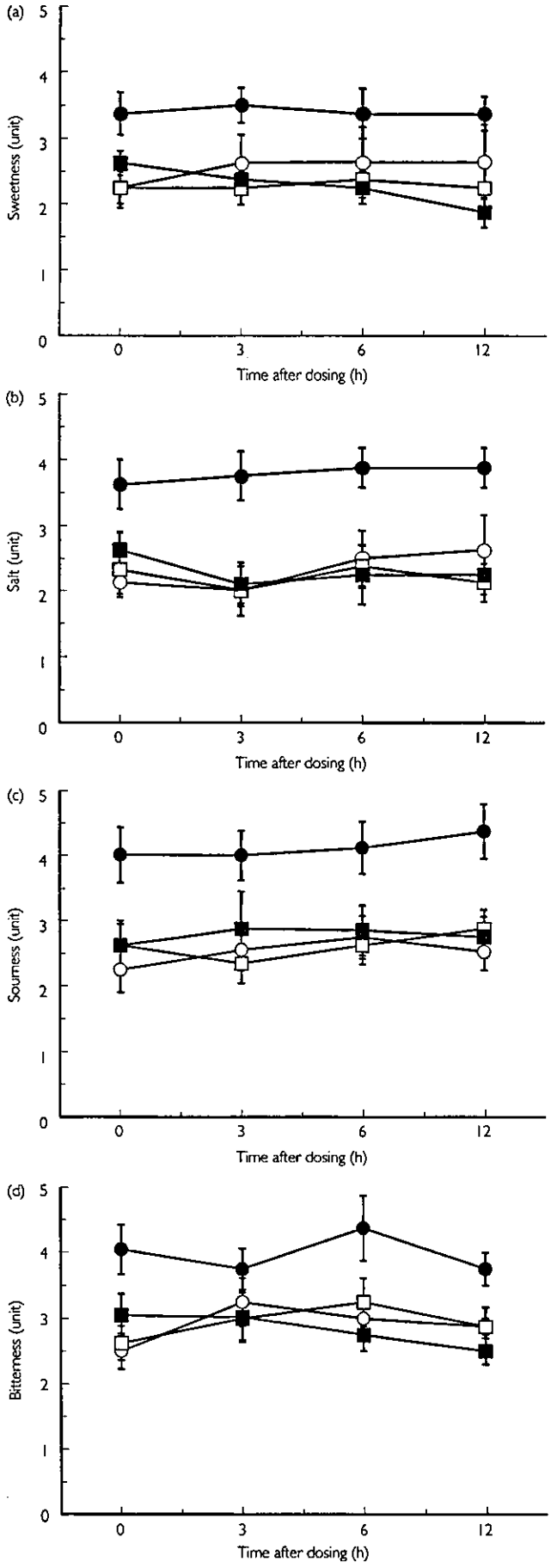

Figure 1

Detection thresholds for tastes using filter-paper disc after repeated dosing of candesartan. Four basic tastes (sweet (a), salty (b), sour (c) and bitter (d)) were evaluated by using chemical solutions before (day 1 + day 23) and at the end of each treatment (day 8 + day 30). Mean ± SEM, $n = 8$. Candesartan: before (○), after (●); Placebo: before (□), after (■)

olds of four different tastes were significantly ($P < 0.05$) worsened after the repeated treatment with candesartan, but not with placebo. Significant ($P < 0.05$) differences were observed at every observation point between the two trials. Mean ± SEM (and 95% CI) scores of four tastes at 24 h after the sixth dosing (i.e. just before the last dosing) of candesartan or vehicle, respectively, were 3.38 ± 0.32 (3.02, 3.74) and 2.63 ± 0.18 (2.18, 3.08) for sweetness, 3.63 ± 0.38 (4.49, 2.77) and 2.63 ± 0.26 (3.27, 1.98) for saltiness, 4.01 ± 0.42 (3.04, 4.98) and 2.61 ± 0.32 (3.35, 1.87) for sourness, 4.01 ± 0.38 (3.22, 4.80) and 2.99 ± 0.33 (2.24, 3.74) for bitterness, respectively. The mean (± SEM) changes of the scores for candesartan and placebo, respectively, were 0.81 ± 0.32 and −0.07 ± 0.21 for sweetness, 0.99 ± 0.39 and 0.07 ± 0.22 for saltiness, 1.35 ± 0.44 and 0.09 ± 0.27 for sourness, 1.02 ± 0.36 and −0.05 ± 0.28 for bitterness. Although the thresholds of sourness in three subjects and bitterness in four subjects were in the abnormal range (i.e. 5 units), the subjects did not notice these changes.

Detection threshold using an electrogustometer is shown in Figure 2. Compared with the observation period, the threshold was significantly ($P < 0.05$) worsened after repeated treatment with candesartan, but not with placebo. However, all values remained within the normal range. Mean ± SEM (and 95% CI) scores of threshold at 24 h after the thirteenth dosing (i.e. just before the last dosing) of candesartan or vehicle were +2.75 ± 0.84 (4.72, 0.73) and −0.25 ± 1.28 (2.02, −2.04), respectively.

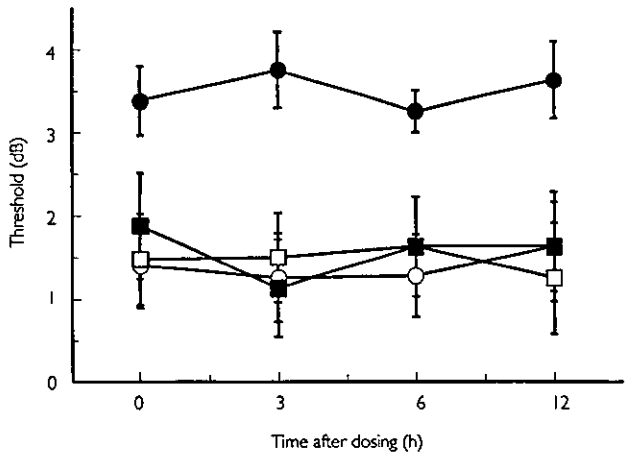

Figure 2
Detection threshold using electrogustometer after repeated dosing of candesartan before (day 1 + day 23) and at the end of each treatment (day 8 + day 30). Mean ± SEM, $n = 8$. Candesartan: before (○), after (●); Placebo: before (□), after (■)

Salivary zinc concentration before (day 1, day 23) and at the end of repeated treatment (day 8, day 30) with candesartan and placebo are shown in Figures 3a and b. There were no significant differences in this parameter between the two trials. No significant differences were observed in serum zinc concentrations between the candesartan and placebo trials (Figure 4). Serum, but not salivary candesartan and M-II were detected after the final dosing of the drug (Figure 5).

Discussion

The filter-paper disc test is semiquantitative, and the detection thresholds for tastes vary slightly among individuals [15]. To avoid such interindividual variation, this

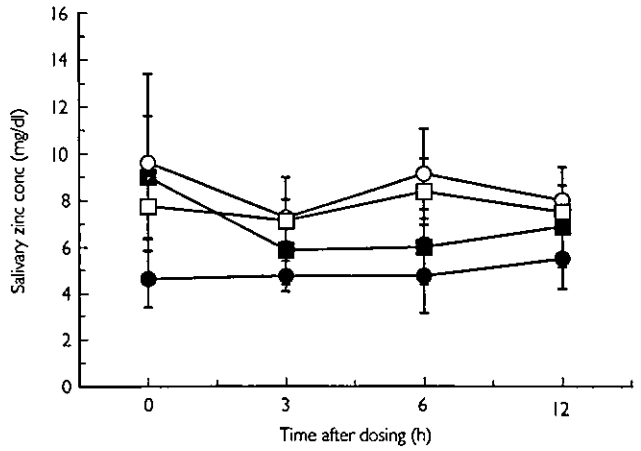

Figure 3
Salivary zinc concentration before (day 1 + day 23) and at the end (day 8 + day 30) of the treatment. Mean ± SEM, $n = 8$. Candesartan: before (○), after (●); Placebo: before (□), after (■)

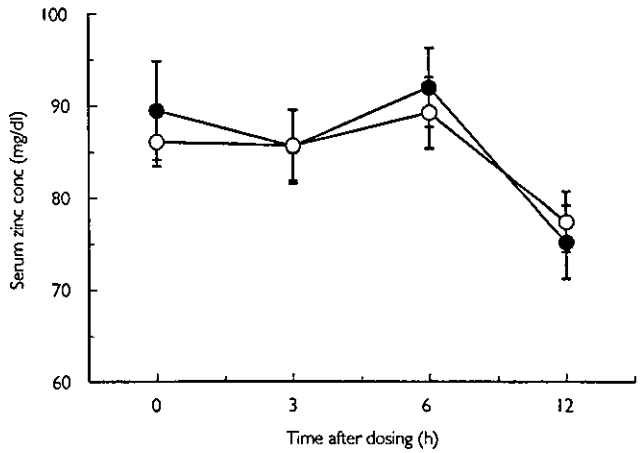

Figure 4
Serum zinc concentration at the end of each treatment. Mean ± SEM, $n = 8$. Candesartan (●), placebo (○)

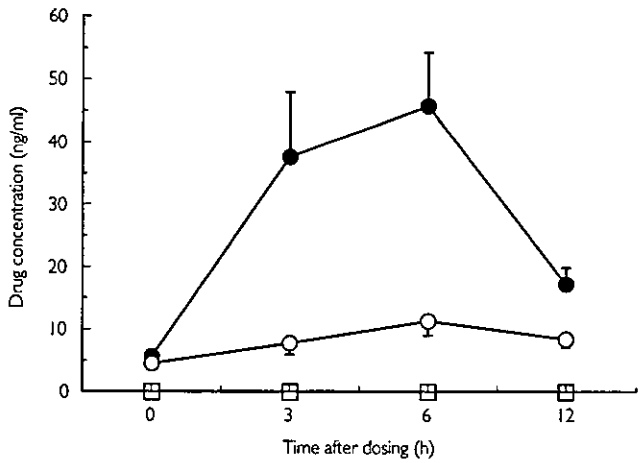

Figure 5

Salivary and serum concentrations of candesartan and M-II after repeated dosing of the drug (4 mg, once daily for 7 days). Mean ± SEM, $n = 8$. Serum candesartan (●), serum M-II (○), salivary candesartan (□). Candesartan and M-II were not detected in salivary fluid at any observation point

study was performed using a randomized, double-blind, placebo-controlled, cross-over design. Detection threshold was also determined by an electrogustometer.

Previous case reports have shown that the patients noticed a metallic taste or burning feeling on the tongue, and lost all four tastes during repeated treatment with losartan [6–8]. Their taste disturbance disappeared within a few weeks after discontinuation of the drug. We evaluated the effect of candesartan on four basic tastes (sweet, salty, sour and bitter) using filter-paper discs. We found that the drug caused subclinical disturbances for these tastes after repeated dosing in healthy subjects. Candesartan-induced taste disturbance was also detected by the electrogustometer. In particular, bitterness and sourness were worsened to a pathological level in almost half the subjects, although they did not notice the disturbance. Based on these findings together with previous observations, we think that taste disturbance is not specific for losartan, but is a class effect of ARBs.

Hypertensive patients noticed the taste disturbance after repeated dosing of losartan and valsartan for 1 week to 3 months [6–9], which indicates that ARB-induced taste disturbance gradually developed. In addition, the present data suggest that this adverse effect persisted for more than 24 h after each dosing of candesartan during repeated treatment. However, the detection thresholds at the observation period (day 1, day 23) of the candesartan and placebo trials did not differ significantly. This indicates that the candesartan-induced taste disturbance was reversible within 2 weeks after the discontinuation of the drug.

A variety of drugs are reported to cause taste disturbance, including thiamazol, D-penicillamine and captopril [16]. Taste disturbance induced by thiamazol and D-penicillamine has been ascribed to the complexing of zinc by these drugs [17] while captopril caused this adverse effect by chelation of zinc [2–4]. Zinc-unrelated taste disturbances have also been reported [2, 3]. In this study, serum and salivary zinc concentrations were not influenced by the repeated dosing of candesartan. Thus, we found no evidence that shows involvement of zinc in candesartan-induced taste disturbance. In salivary fluid, both bound and unbound fractions of zinc were measured. It is therefore uncertain whether the bound fraction of zinc with metalloprotein in the salivary fluid was changed by the drug. Recent advances in molecular biology have identified receptors and ion channels on taste cells. Sweet and bitter taste receptors are the proteins that couple with G-proteins [18, 19]. Coupling and uncoupling to G-protein causes 'taste-on' and 'taste-off' [2]. The taste receptors have seven transmembrane domains [18, 19]. Angiotenisin II receptor, which is the target molecule of ARB, also belongs to the same category of receptor [20]. It is possible that ARBs are secreted in salivary fluid, which in turn, bind with taste receptors and consequently disturb the sense of sweet and bitter tastes. On the other hand, salt and sour tastes are elicited by ion channels (salt; amiloride-sensitive epithelial Na channel, sour; amiloride-sensitive epithelial Na channels and H^+-activated cation channels [18, 19]. In this case, salivary ARBs might plug these channels and disturb salt and sour tastes. Although salivary candesartan and its metabolite were not detected in this study, the above-mentioned hypothesis remains to be determined.

In summary, this study shows that repeated dosing with candesartan subclinically reduces the sensitivity of basic tastes. The effect was reversible after discontinuation of the drug within 2 weeks. Because similar events are reported for losartan and valsartan in the clinical setting, taste disturbance might be a class effect of ARBs. Similar events are reported for ACE-I with unknown mechanisms except for captopril. Further studies are needed to determine the frequency and grade of taste disturbance for each ARB.

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Chronopharmacology of oxacalcitriol in 5/6 nephrectomized rats

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Abstract

We previously reported on the merits of the chronopharmacological effects of 1,25(OH) 2 vitaminD3 in 5/6 nephrectomized rats (Tsuruoka et al, Life Scineces 2002; 71: 1809–1820). In this study, the chronopharmacological effect of 22-oxacalcitriol (OCT), a newly developed active vitaminD3 analogue with less calcemic activity, was evaluated by a single and repeated dosing of the drug. The 5/6 nephrectomized animals were kept in rooms with a 12-h light/dark cycle. Single (12.5 μg/kg, i.v.) and repeated (5 μg/kg, i.v. three times a week for 12 weeks) dosing of OCT or vehicle was given at either 2 hours after lights on (2HALO) or 14 hours after lights on (14HALO). The severity of hypercalcemia and hyperphosphatemia was significantly milder when the drug was given at 14HALO. Serum concentrations of total OCT and albumin of the 2HALO and 14HALO trials did not differ significantly. The decrease of parathyroid hormone concentration was greater in the 14HALO trial while the increase in urinary ratio of Ca to creatinine was greater in the 2HALO trial. The suppression of urinary deoxypyridinoline excretion, an index of bone resorption capacity of osteoclast, and the increase in bone density of both femurs were greater in the 14HALO trial. These results suggest that the adverse reactions of OCT were ameliorated and its efficacy was enhanced after dosing of the drug at 14HALO. Chronopharmacological differences of OCT were more prominent than those seen with other vitamin D analogues. Dosing-time-dependent variation in the sensitivity of the drug to osteoclast were involved in the mechanisms of these events.

Keywords: 22-oxacalcitriol; Renal osteodystrophy; Chronotherapy; Hypercalcemia

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Introduction

Although active vitamin D analogues are widely used for the treatment of osteoporosis and secondary hyperparathyroidism, the drug-induced hypercalcemia and hyperphosphatemia sometimes limit their efficacy (Parfitt, 1988). It is well known that serum Ca and P concentrations show diurnal changes in both humans (Tsuruoka et al., 1999) and rats (Shinoda and Seto, 1985; Shinoda and Stern, 1992). We previously reported that hypercalcemia and hyperphosphatemia induced by 1,25(OH)2vitaminD3, an active vitamiD3, could be reduced by administration of the drug at night in 5/6 nephrectomized rats (Tsuruoka et al., 2002), in patients with secondary hyperparathyroidism (Tsuruoka et al., 1999, 2003), and in an animal model of osteoporosis (Tsuruoka et al., 2001). We have also shown that the efficacy of vitaminD3 therapy can be enhanced by administering the drug at night in the above mentioned animal models and human trials (Tsuruoka et al., 2001, 2002, 2003).

Compared to 1,25(OH)2 vitaminD3, 22-oxacalcitriol (OCT or maxacalcitriol), a new analogue of vitaminD3, was reported to have less hypercalcemic effect with similar efficacy (Brown et al., 1993; Kubrusly et al., 1993; Farach-Carson et al., 1993). This compound is now used for the treatment of secondary hyperparathyroidism and osteoporosis in clinical situations. However, it remains to be determined whether OCT also possesses chronopharmacological effects. To address this issue, the present study was undertaken to evaluate a dosing-time dependent change in the effects of OCT in 5/6 nephrectomized rats. We obtained some chronopharmacological profiles in its efficacy and adverse reactions, which were compared with our previous results using other vitamin D analogues.

Methods

Animals

Eight week old male Wistar rats (Japan SLC, Shizuoka, Japan, n = 40) were used in this study. They had free access to standard rat chow (CE-2, containing 1.18% Ca and 2.5 IU/g vitamin D3, Japan Clea Co.Ltd., Tokyo, Japan) and distilled water until the end of the study. Two animals were kept in a single cage. Under pentobarbital anesthesia, 5/6 nephrectomy was performed by ligation of renal artery branches supplying two-thirds of left kidney, followed by a right unilateral nephrectomy 7-10 days later (Tsuruoka et al., 2002; Sugimoto et al., 1999). After 8 weeks, creatinine clearance was measured and the animals were divided into four groups. No significant differences in creatinine clearance or body weight were observed among the groups.

The animals were kept in two specific pathogen-free rooms with a 12-h light-dark cycle and diverse lighting schedules, (Yamauchi et al., 1998; Tsuruoka et al., 2000, 2001, 2002; Nishiki et al., 2003). In room 1, lights were on at 0700 h and off at 1900 h at a local time. In room 2, lights were on at 1900 h and off at 0700 h. The temperature and humidity in the rooms were maintained automatically. The rooms were maintained at about 23 °C and light intensity in the room was 1500 lux. Two groups were kept in room1 while the other two groups were kept in room 2 until the end of the study. It has been reported that most physiological parameters, such as neuronal, humoral, motor, and behavioral functions, are completely resynchronized within two weeks after changing lighting schedules (Turek, 1985; Mrosovsky and Salmon, 1987; Takahashi and Zatz, 1982; Takamure et al., 1991); this maneuver is well accepted

in the fields of chronobiology and chronopharmacology. The following experiments were conducted in accordance with Jichi Medical School Guide for Laboratory Animals.

Experimental design

Single dosing study

On the experimental day, chow was removed and the animals were placed in other cages to measure body weight about one hour before dosing with the drug. 22-Oxacalcitriol (OCT or maxacalcitriol, 12.5 μg/kg, generous gift from Chugai Pharmaceutical Co.Ltd, Tokyo, Japan) or vehicle was injected into a tail vein 2 hours after lights on (2HALO) and 14 hours after lights on (14HALO) (i.e. 0900 h at a local time).

Group 1: 2HALO with OCT, n = 10
Group 2: 2HALO with vehicle alone, n = 10
Group 3: 14HALO with OCT n = 10 and
Group 4: 14HALO with vehicle alone, n = 10.

The dose, that increased serum Ca concentrations, was selected on the basis of our preliminary study. Venous blood samples were taken before and 2, 4, 6, 8, 12, 18 and 24 h after dosing. Serum samples were frozen and kept at −80 °C until the assay. These protocols were performed after a two-week acclimatization period in a cross-over fashion.

Repeated dosing study

The 5 μg/kg of OCT or vehicle was injected into a tail vein at 2 HALO and 14HALO (i.e. 0900 h at a local time) three times a week for 12 weeks. Body weight was measured twice weekly until the end of the study. Blood samples were obtained at 0900h (24 hours after the last dose of the drug) just before and 4, 8 and 12 weeks after the initiation of administration. Four-hour urine specimens were collected just before and 4, 8 and 12 weeks after the start of the study. For the collection of urine, deionized water (3% of body weight) was given by gastric gavage 30 min after dosing with OCT or vehicle, and the animals were separately placed in metabolic cages for 4 hours (Tsuruoka et al., 2001). Urine collection was performed one day before blood sampling. These procedures were similar to those in our previous paper (Tsuruoka et al., 2002) except that drug effects were evaluated. Both serum and urine were stored at −80 °C until the assay. Both femurs were obtained at the end of the study and frozen at −80 °C.

Assays

Serum and urine Ca concentrations were measured by the orthocresolphthalein complexone method (Connerty and Briggs, 1966) and the ammonium molybdate method (Drewes, 1972) with an auto-analyzer, respectively. Creatinine concentration was measured by the modified Jaffe's reaction with an auto-analyzer. Serum albumin was measured by an enzyme-linked immunosorbent assay kit (Panatest; Wako chemical, Osaka, Japan, (Sugimoto et al., 2002). Serum OCT concentration was measured by liquid chromatography-mass spectrometry (Ishigai et al., 1998). Detection limit was 10 pg/ml. Serum parathyroid hormone (PTH) concentration was measured by an immunoradiometric assay (rat PTH

IRMA kit, Immutopics, Inc. SanClemente, CA, USA). Its normal range is 10–40 pg/ml (Tsuruoka et al., 2001, 2002). Urine deoxypyridinoline, which was used as an index of bone resorption, was measured by reverse-phase high-performance liquid chromatography (Seyedin et al., 1993), and its excretion is expressed as a ratio to creatinine concentration.

Bone density of femurs was determined by dual-energy X-ray absorption (DEXA, DCS-600A, Aloka, Japan). The scan was performed every 2 mm along the axis of the bone from the proximal end. Usually 14-17 scans were made for each bone. An average of the first 3 proximal scans, 4 scans of middle part, and last 3 scans are termed "proximal", "medial", and "distal", respectively. The average of all scans is termed "whole". "Medial" is exclusively cortical bone and "distal" is rich in cancellous bone (Shen et al., 1995; Tsuruoka et al., 2001, 2002).

Statistics

All data are presented as the means ± S.E. Statistical analysis was performed by analysis of variance or Student's t-test as appropriate. P values less than 0.05 was regarded as significant.

Results

Single dosing study

Fig. 1 shows the changes in serum Ca concentrations during the two different dosing schedules. When OCT was injected at 2HALO, the serum Ca concentration gradually increased for six hours and then decreased (Fig. 1A). There was no significant difference between the OCT and vehicle trials at 12 hours after dosing. When the drug was injected at 14HALO, serum Ca concentrations slightly but significantly increased while such differences disappeared 8 hours after dosing (Fig. 1B). In the vehicle groups, serum Ca concentrations showed a diurnal change, which is compatible with previous observations (Tsuruoka et al., 2000, 2001, 2002; Nishiki et al., 2003). The difference in the area under the concentration-curve between OCT and vehicle trials was significantly ($P < 0.01$) greater in the 2HALO trial (2HALO; 6.3 ± 1.3 mg.hrs/dl, 14HALO;1.8 ± 0.3 mg.hrs/dl). Because ionized Ca concentrations are affected by the serum albumin concentration, this parameter was simultaneously measured. We found that serum albumin was not influenced by the injection of OCT (data not shown). The area under the concentration-curve of serum albumin was 70.6 ± 3.3 and 70.2 ± 3.8 mg .h/dl (OCT and vehicle, respectively) in the 2 HALO trial and 70.4 ± 3.2 and 69.8 ± 2.7 mg .h/dl (OCT and vehicle, respectively) in the 14HALO trial. This finding indicates that serum total as well as ionized Ca concentrations were actually increased by the drug. We also confirmed the diurnal change in serum albumin concentrations in the vehicle group, which was reported previously (Tsuruoka et al., 2000).

Fig. 2 shows the changes in serum P concentrations during the two different dosing schedules. When OCT was injected at 2HALO, serum P concentrations gradually increased for six hours and then decreased (Fig. 2A). There was a significant difference between the OCT and vehicle trials at 18, but not at 24 hours after dosing. When the drug was injected at 14HALO, serum P concentration increased and was significantly higher than that of vehicle up to 8 hours. In the vehicle groups, serum P concentration showed a diurnal change, which is compatible with previous observations (Tsuruoka

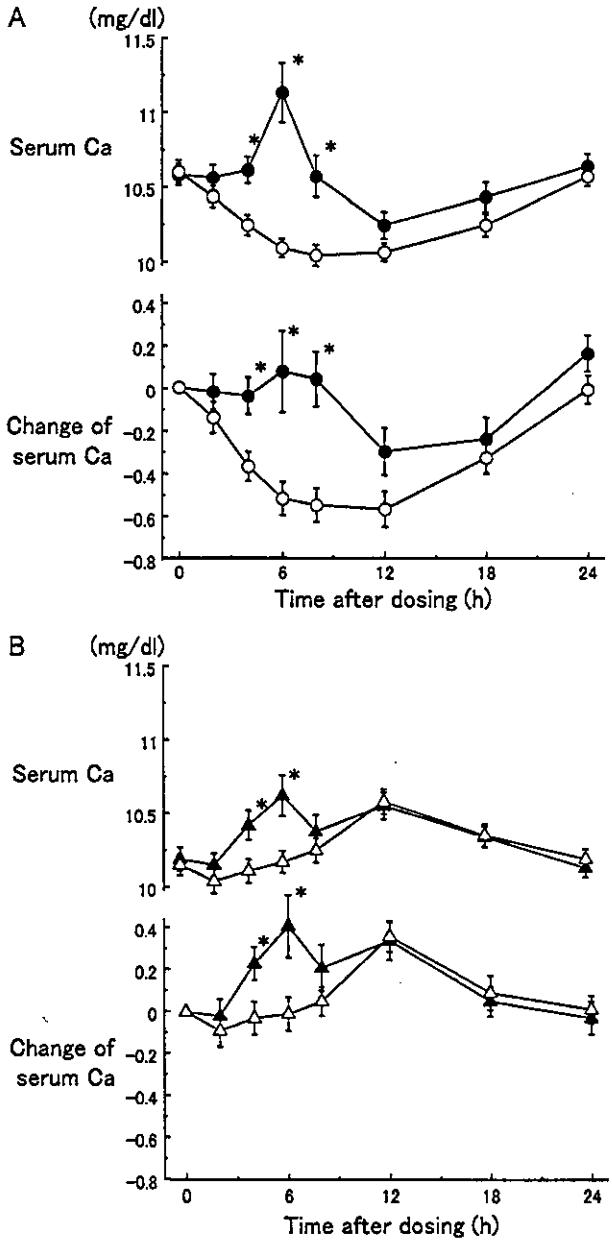

Fig. 1. Serum Ca concentration after a single dosing of OCT or vehicle at 2HALO (A) and 14HALO (B) in 5/6 nephrectomized rats. Mean ± S.E., n = 40 in each. *P < 0.05 vs. vehicle. ● OCT (2HALO), ○ vehicle (2HALO) ▲ OCT (14HALO), △ vehicle (14HALO).

et al., 2000, 2001, 2002). The difference in the area under the concentration-curve between the OCT and vehicle trials was significantly (P < 0.01) greater in the 2HALO trial (2HALO; 33.7 ± 3.8 mg.hrs/dl, 14HALO;11.8 ± 1.7 mg.hrs/dl).

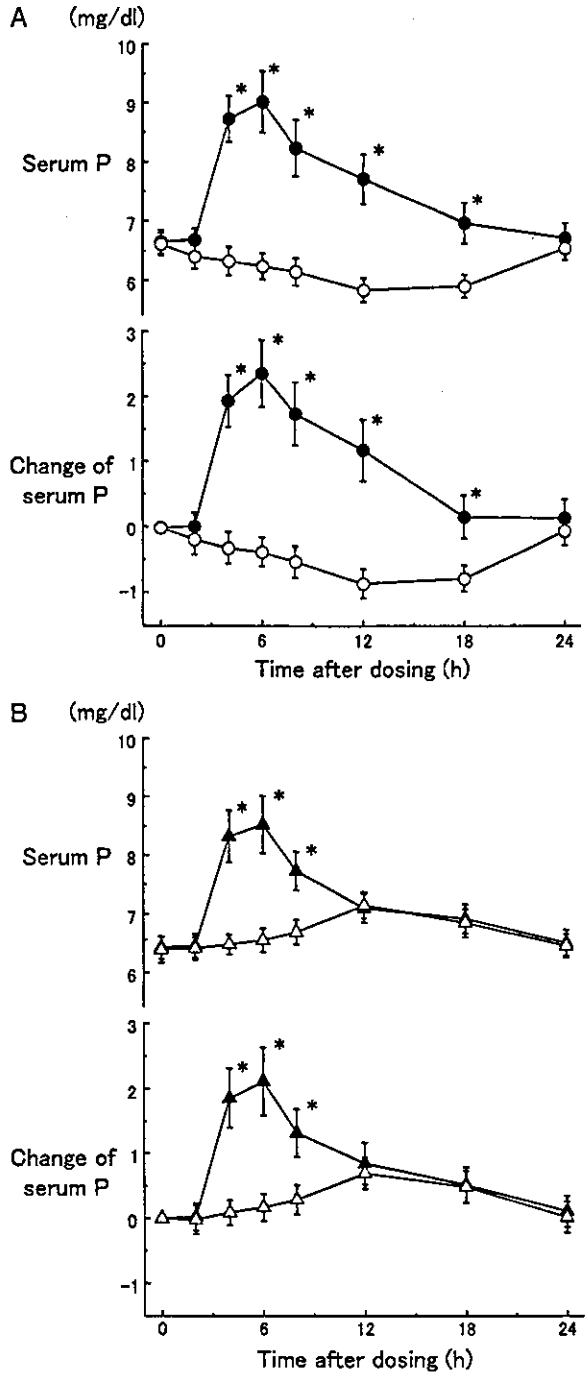

Fig. 2. Serum P concentration after single dosing of OCT or vehicle at 2HALO (A) and 14HALO (B) in 5/6 nephrectomized rats. Mean ± S.E., n = 40 in each. *P < 0.05 vs. vehicle. ● OCT (2HALO), ○ vehicle (2HALO). ▲ OCT (14HALO), △ vehicle (14HALO).

Repeated dosing study

Changes in body weight and creatinine clearance

All animals completed the study without any obvious symptoms. The changes in body weight and creatinine clearance (Ccr) are shown in Fig. 3. There was a slight but significant ($P < 0.05$ vs. pre in each) decrease in body weight during the study in all groups. The decrease in this parameter of 4 groups did not differ significantly. Ccr decreased gradually during the study without significant differences between the groups. The changes of these parameters were comparable with our previous findings in 5/6 nephrectomized rats (Tsuruoka et al., 2002).

Serum concentrations of inorganic Ca, P, albumin, PTH and OCT

Serum inorganic Ca and P concentrations before the study were significantly different between the 2HALO and 14HALO trials (Fig. 4). These data are compatible with our previous data (Tsuruoka et al., 2000, 2001, 2002) and data in the literature for humans and rats(Shinoda and Seto, 1985; Calvo et al., 1991). Treatment with OCT significantly increased serum Ca concentrations in the 2HALO but not in the 14HALO trial (Fig. 4). Mean change in the serum Ca concentration at the end of the study was 0.4 ± 0.1 mg/dl in the 2HALO and 0.1 ± 0.1 mg/dl in the 14HALO trial ($P < 0.05$). Vehicle alone reduced Ca concentrations in both 2HALO and 14HALO trials. Serum P concentrations increased in all groups and the increment was greater in the 2HALO trial (Fig. 4). The mean change in serum P concentration at the end of the study was 1.3 ± 0.3 mg/dl in the 2HALO and 0.4 ± 0.3 mg/dl in the 14HALO trial. Serum albumin concentration, which was measured using

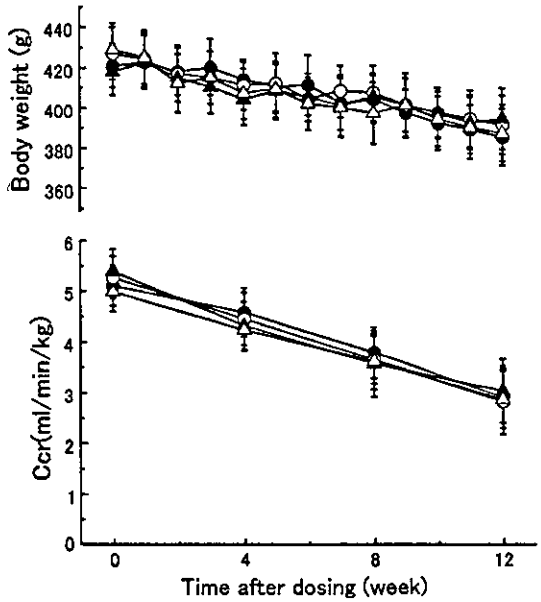

Fig. 3. Body weight and creatinine clearance (Ccr) during a repeated dosing of OCT or vehicle at 2 and 14HALO in 5/6 nephrectomized rats. Mean ± S.E., n = 10 in each. ● OCT (2HALO), ○ vehicle (2HALO). ▲ OCT (14HALO), △ vehicle (14HALO).

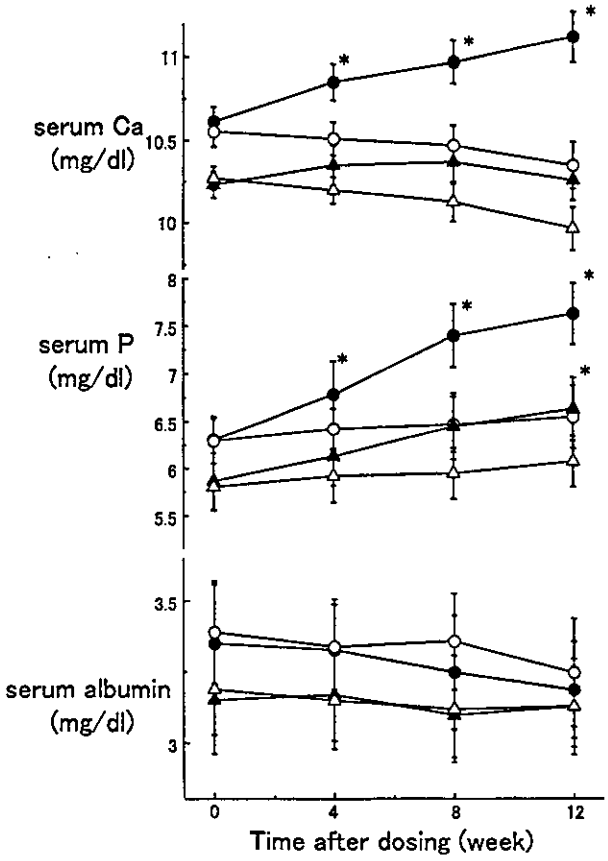

Fig. 4. Serum concentrations of Ca, P and albumin during a repeated dosing of OCT in 5/6 nephrectomized rats. Mean ± S.E., n = 10 in each. *P < 0.05 vs. each control. ● OCT (2HALO), ○ vehicle (2HALO). ▲ OCT (14HALO), △ vehicle (14HALO).

same specimens, was not changed by OCT in the 2HALO and 14HALO (Fig. 4). We also evaluated the trough concentrations of serum OCT, which did not differ significantly between the groups (Fig. 5). Serum PTH concentrations were increased by the vehicle, and reduced following OCT at both dosing-times (Fig. 5). The decrease was significantly greater in the 14HALO trial. Mean decrease of PTH concentration after OCT treatment was 21 ± 16 pg/ml in the 2HALO and 60 ± 14 pg/ml in the 14HALO trial.

Urinary excretions of Ca, P, creatinine and DPD

The urinary ratio of Ca to creatinine (u-Ca/u-Cr) at 4 hours is shown in Fig. 6. The basal value was greater in the 2HALO than in the 14HALO trials, which was consistent with our previous results (Tsuruoka et al., 2002). OCT increased the ratio in the 2HALO but not in the 14HALO trial. The amount of Ca in the 4-hour urine showed a similar tendency, which was also comparable with our previous study (data not shown) (Tsuruoka et al., 2002).

The u-P/u-under basal conditions was also greater in the 2HALO than in the 14HALO, and was increased by the drug (Fig. 6). However, there was no significant difference in the drug-related changes in the ratio between the 2HALO and the 14HALO trials (P = 0.08). Urinary excretion of deoxypyridino-