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Chronopharmacology of oxacalcitriol in rat model of osteoporosis

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Abstract

We have previously reported the merits of chronopharmacological effect of 1-α(OH) vitamin D3 in aged stroke-prone spontaneously hypertensive rat (SHRSP), a model of osteoporosis [Eur. J. Pharmacol. 428 (2001) 283.]. In this study, the chronopharmacological effect of 22-oxacalcitriol, a newly developed active vitamin D3 analogue with less calcemic activity, was evaluated by a single and repeated dosing of the drug in aged SHRSP. Animals (7 months old) 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 22-oxacalcitriol or vehicle was given at either 2 h after lights on (2HALO) or 14 h after lights on (14HALO). The severity of adverse reactions such as the changes of body weight, hypercalcemia and hyperphosphatemia, was significantly mild when the drug was given at 14HALO. Especially, the increase of serum Ca concentration was not detected at 14HALO trial. Serum concentrations of total (protein-bound and unbound) 22-oxacalcitriol and albumin (a major binding protein of the drug) of the 2HALO and 14HALO trials did not significantly differ. The decrease of parathyroid hormone (PTH) concentration was greater in the 14HALO trial while the increase in urinary ratio of Ca to creatinine was greater in the 2HALO trial. The increase in bone density of both femurs at the end of the study was greater in the 14HALO trial. The suppression of urinary excretion of deoxypyridinoline, an index of bone resorption capacity of osteoclast, was greater in the 14HALO trial, which indicates that the efficacy of 22-oxacalcitriol for suppressing bone resorption might vary with the dosing time. This is the first study to show the dosing-time-dependent changes in the efficacy and toxicity of 22-oxacalcitriol in the animal model of osteoporosis. Chronopharmacological differences seem to be more prominent than those of other vitamin D analogues. To use 22-oxacalcitriol at an adequate timing might provide better efficacy and safety than other vitamin D3 analogues for the treatment of osteoporosis.
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Keywords: 22-Oxacalcitriol; Osteoporosis; Chronotherapy; Hypercalcemia

1. Introduction

Although active vitamin D analogues are widely used for the treatment of osteoporosis and secondary hyperparathyroidism, the drug-related hypercalcemia and hyperphosphatemia sometimes limit its 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 have previously reported that hypercalcemia and hyperphosphatemia by an active vitamin D3 can be diminished by administration of the drug at night in aged stroke-prone spontaneously hypertensive rats (SHRSP), a model of osteoporosis (Tsuruoka et al., 2001), and patients with secondary hyperparathyroidism (Tsuruoka et al., 1999, 2003). We have also reported that the efficacy of vitamin D3 therapy can be enhanced by the drug administration at night in aged SHRSP (Tsuruoka et al., 2001), 5/6-nephrectomized rats (Tsuruoka et al., 2002), and patients with secondary hyperparathyroidism (Tsuruoka et al., 2003).

Compared to 1,25(OH)2 vitamin D3, the 22-oxacalcitriol (or maxacalcitriol), a new analogue of vitamin D3, is reported to have a 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 situation. However, it remains to be determined whether 22-oxacalcitriol also possesses chronopharmacological effect. It was a purpose of this study to evaluate a dosing-time-dependent change in the effects of 22-oxacalcitriol in aged SHRSP, a rat model of osteoporosis. We obtained some chronopharmacological profiles in its efficacy and adverse reactions, which were compared with our previous results using other vitamin D analogues.

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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

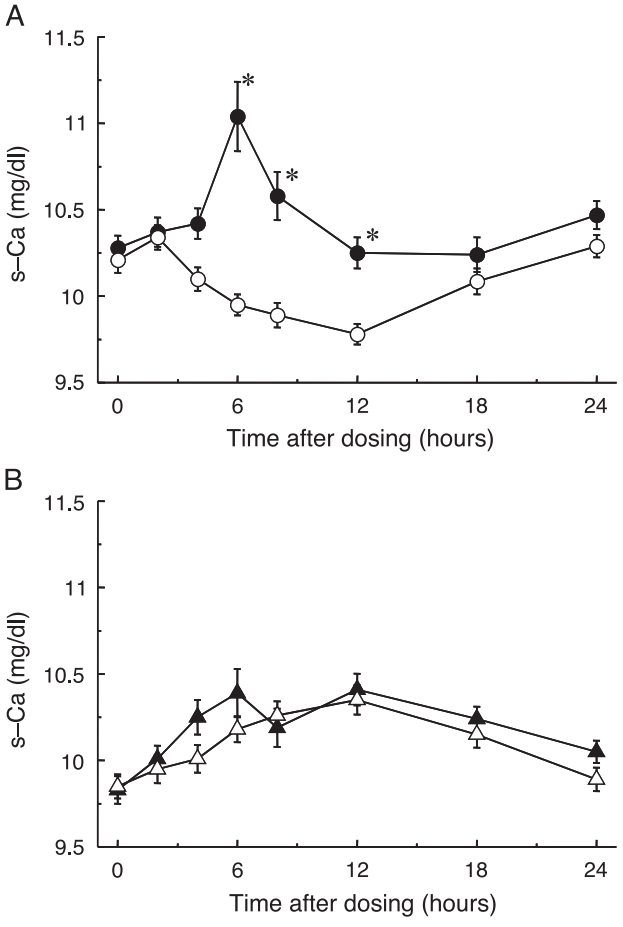

Fig. 1. Serum Ca concentration (s-Ca) after a single dosing of 22-oxacalcitriol or vehicle at 2HALO (A) and 14HALO (B) in aged SHRSP. Mean ± S.E., $n = 10$ in each. $^*P < 0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

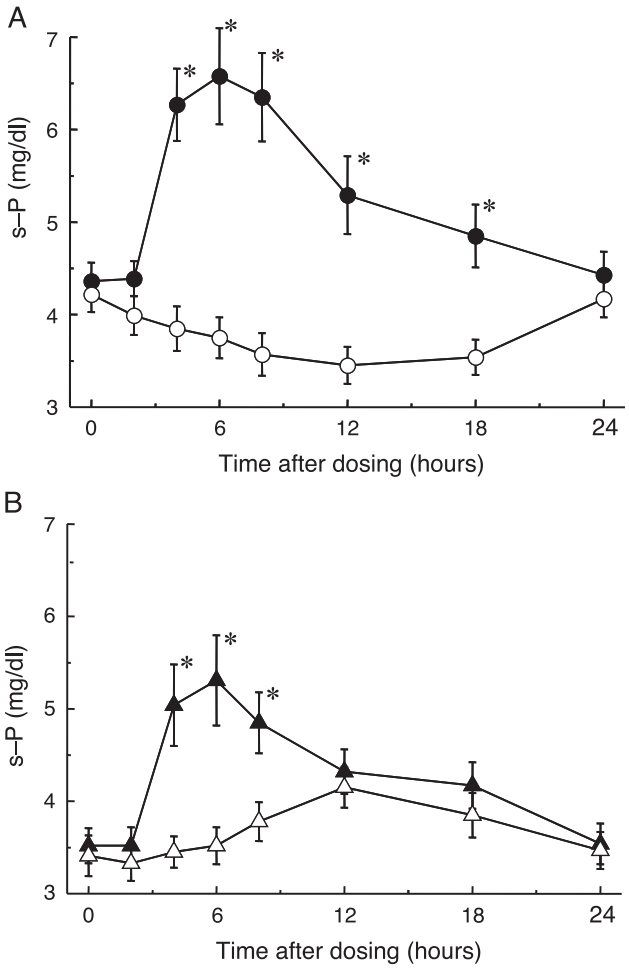

Fig. 2. Serum P concentration (s-P) after a single dosing of oxacalcitriol or vehicle at 2HALO (A) and 14HALO (B) in aged SHRSP. Mean ± S.E., $n = 10$ in each. $^*P < 0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

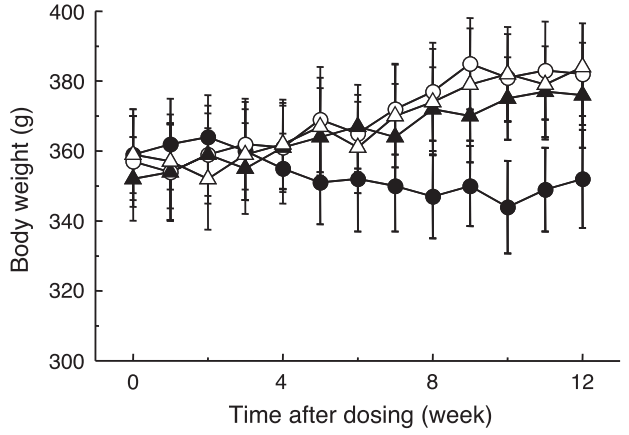

Fig. 3. Body weight during a repeated dosing of oxacalcitriol or vehicle at 2 and 14HALO in aged SHRSP. Mean ± S.E., $n = 10$ in each. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

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-

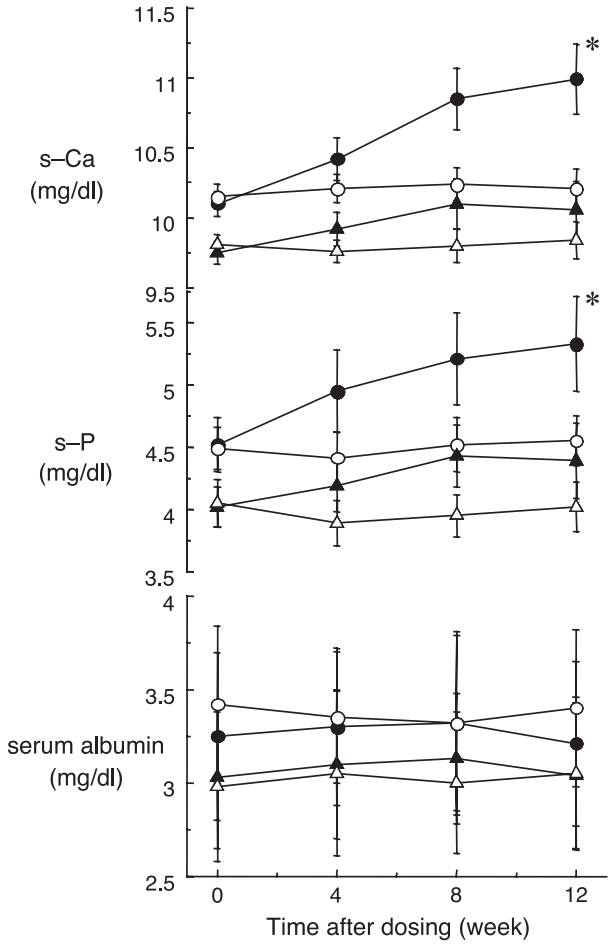

Fig. 4. Serum concentrations of Ca, P and albumin during a repeated dosing of oxacalcitriol in aged SHRSP. Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

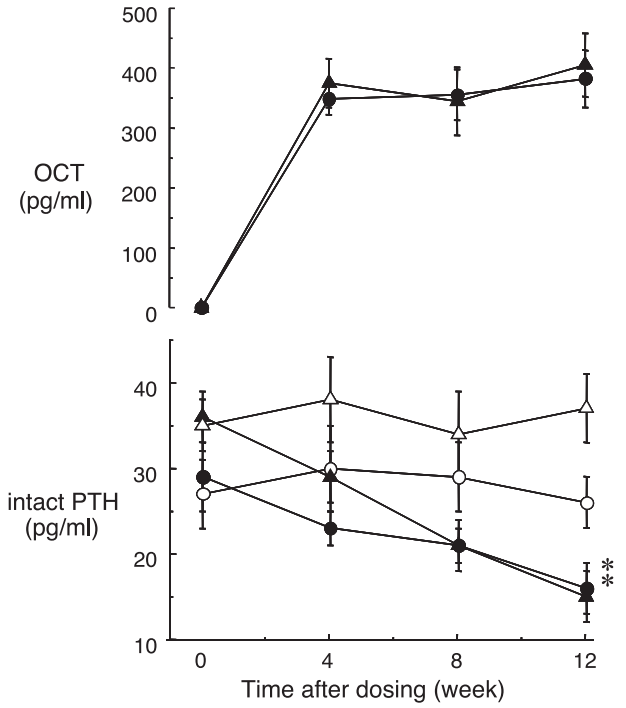

Fig. 5. Serum concentrations of oxacalcitriol and PTH during a repeated dosing of oxacalcitriol in aged SHRSP. Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

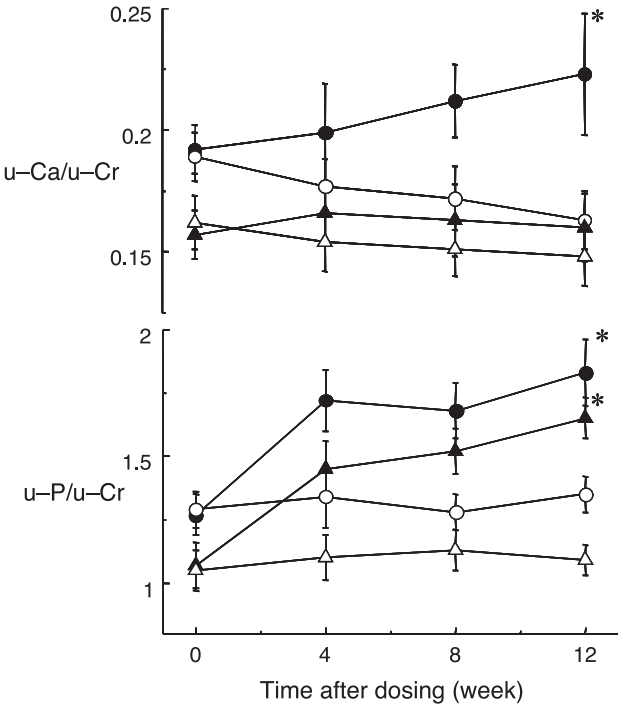

Fig. 6. The ratio of urinary Ca to creatinine (u-Ca/u-Cr) and urinary P to creatinine (u-P/u-Cr) during a repeated dosing of oxacalcitriol in aged SHRSP. Mean ± S.E., $n=10$ in each. *$P<0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ oxacalcitriol (14HALO), △ vehicle (14HALO).

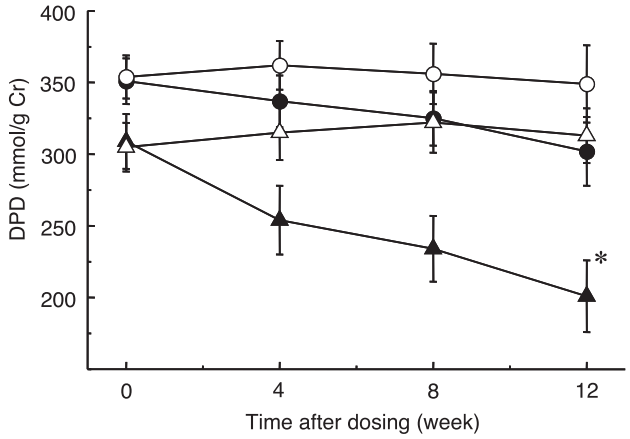

Fig. 7. Urinary deoxypyridinoline excretion during a repeated dosing of oxacalcitriol in aged SHRSP. Mean ± S.E., $n = 10$ in each. *$P < 0.05$ vs. each control. ● Oxacalcitriol (2HALO), ○ vehicle (2HALO). ▲ Oxacalcitriol (14HALO), △ vehicle (14HALO).

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

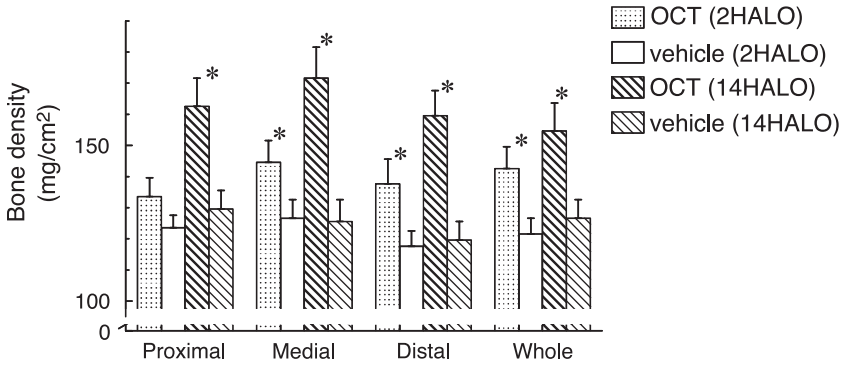

Fig. 8. Bone density of femur at the end of the study, Mean ± S.E., $n = 10$ in each. *$P < 0.05$ vs. vehicle, #$P < 0.05$ vs. morning.

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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