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Dosing time-dependent variation in the hypocalcemic effect of calcitonin in rat

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Abstract

Dosing time-dependent variation in the hypocalcemic effect of salmon calcitonin was examined in rats under a 12-h light–dark cycle. In both a single-dosing study with normal rats and a repeated-dosing study with hypercalcemic rats (induced by chronic vitamin-D dosing), we consistently observed that the hypocalcemic effect of calcitonin was greater when the drug was given at 14 h after lights on than that at 2 h after lights on. The reduction in urinary deoxypyridinoline excretion, a marker of bone resorption, was also greater when calcitonin was given at 14 h after lights on. Urinary excretion of Ca was not affected by the drug. Pharmacokinetic profiles of calcitonin after a single dosing did not differ between the two trials. These results indicate that the hypocalcemic effect of calcitonin is greater after dosing in the early dark phase (14 h after lights on) than after dosing in the early light phase (2 h after lights on). A time-dependent variation in the sensitivity to the drug of osteoclasts, but not renal tissues, may be involved in the mechanism of this event.

Keywords: Calcitonin; Hypocalcemia; Chronopharmacology; (Rat)

1. Introduction

Daily rhythmic fluctuations are reported in several parameters of Ca metabolism, such as serum Ca and phosphate concentration. These variables have a peak in the "light phase" and a trough in the early "dark phase" in both humans (Calvo et al., 1991; Tsuruoka et al., 1999) and rats (Shinoda and Seto, 1985; Tsuruoka et al., 2000) while behavioral activity is high during the "light phase" in humans and in the "dark phase" in rats. The balance of bone formation and resorption is altered by light, which subsequently affects the rhythm of Ca^{2+} metabolism (Shinoda and Stern, 1992; Silver et al., 1996). It is also well known that some drugs, such as vitamin D, cause hypercalcemia. We have previously reported that changing the dosing time affects the magnitude of hypercalcemia induced by vitamin D_3 in both human and animal models (Tsuruoka et al., 1999, 2000, 2001, 2002).

Calcitonin is often used for the treatment of osteoporosis (Stevenson and Evans, 1981) and hypercalcemia (Finkelstein, 2001). However, to our knowledge, it is uncertain whether the hypocalcemic effect of calcitonin depends on its dosing time. This study was undertaken to address this issue. We also examined the changes in renal Ca excretion and bone resorption to evaluate potential mechanisms for the chronopharmacological effect of calcitonin. Because we found a dosing time-dependent change in the hypocalcemic effect of calcitonin in a single-dosing study, we examined whether this effect persisted during repeated dosing in animals with chronic hypercalcemia.

2. Methods

2.1. Animals

Ten-week-old male Wistar rats (Japan SLC, Shizuoka, Japan) were used. They had free access to standard chow (CE-2, containing 1.18% Ca and 2.5 IU/g vitamin D_3,

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Japan Clea, Tokyo, Japan) and deionized water until the end of the study. The animals were kept in two specific-pathogen-free rooms with different lighting schedules in the vivarium of our medical school (Tsuruoka et al., 2000, 2001, 2002). In room 1, lights were on at 0700 h and off at 1900 h at 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 animals were randomly divided into two groups, and one of the groups was moved from room 1 to room 2. After an equilibration period of more than 2 weeks in the new room, the experiments were started. It is reported that most physiological parameters in animals, such as neuronal, humoral, motor, and behavioral functions, are completely re-synchronized within 2 weeks after changing lighting schedules (Mrosovsky and Salmon, 1987; Takamure et al., 1991; Turek, 1985), and this maneuver is well accepted in the fields of chronobiology and chronopharmacology. Two animals were kept in a cage. 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 in normal rats

On the experimental day, chow was removed and the animals ($n = 10$) were placed in other cages to measure body weight (BW) about 1 h prior to drug dosing. Salmon calcitonin (Sigma, 10 m U/250 g BW) or vehicle (0.1 ml of saline) was injected in a tail vein at 2 and 14 h after lights on (i.e. 0900 h at local time). The dose, which decreased serum Ca concentration, was selected on the basis of our preliminary study. Venous blood samples were taken before and at 0.5, 1, 2, 4, 8, 12 and 24 h after dosing. For the collection of urine samples, 3% body weight of deionized water was orally given to the animals at 30 min after dosing of the drug or vehicle, and each animal was placed in a metabolic cage for 6 h. Serum and urine samples were frozen and kept at -80 °C until the assay. These protocols were performed after a 2-week acclimatization period in a crossover fashion.

2.2.2. Repeated-dosing study in vitamin D_3-induced hypercalcemic rats

Animals were randomly divided into four groups ($n = 10$ in each). Two groups were kept in room 1 while the other two groups were in room 2. An animal model of hypercalcemia was established by daily intramuscular injection of 2 μg of 1,25-dihydroxy vitamin D_3 for 2 weeks (Raue et al., 1984). After injection of vitamin D_3 for 1 week, calcitonin (5 m U/250 g BW) or vehicle was injected daily in the tail vein at 2 or 14 h after lights on for 7 days.

Blood samples were obtained once daily just before the injection of the drug from the fifth day after vitamin D therapy (day 2) until the end of the study (day 7). Urine was also collected on the last day of the experiment for 6 h following oral challenge with deionized water (3% of body weight).

2.3. Assays

Serum and urine Ca was measured by an orthocresolphthalein complex method. Creatinine concentration was measured by the modified Jaffe's reaction with an auto-analyzer. Serum total protein was measured by Biuret's method. Serum salmon calcitonin concentration was measured by radioimmunoassay using an antiserum at final dilution of 1:20 000, supplied by Medical System, Genova, Italy (Tarquini et al., 1988). We confirmed that there was no cross-reaction with human/rat calcitonin. Urine deoxypyridinoline, 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.

2.4. Statistics

All data are presented as mean ± S.E. Statistical analysis was performed by analysis of variance or Student's *t*-test as appropriate. *P* values less than 0.05 were 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 calcitonin was injected at 2 h after lights on, serum Ca concentration gradually decreased for 4 h and then went up. There was no significant difference between the calcitonin and vehicle trials at 12 h after dosing. When the drug was injected at 14 h after lights on, serum Ca concentration greatly decreased and the hypocalcemic effect persisted up to 18 h after dosing. In the vehicle groups, serum Ca 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 calcitonin and vehicle trials was significantly ($P < 0.01$) higher in the 14 h after lights on trial (20.5 ± 1.2 and 8.6 ± 0.8 mg h/dl, 14 and 2 h after lights on, respectively).

Because ionized Ca concentration is affected by serum total protein concentration, serum total protein was also measured. This variable was measured at the same times as the serum Ca level, and it was not influenced by the injection of calcitonin (data not shown). The area under the concentration curve of serum protein was 175.2 ± 6.9 and 167.4 ± 7.5 mg h/dl (calcitonin and vehicle, respectively) in the 2 h after lights on trial and 177.4 ± 7.3 and 175.2 ± 7.7 mg h/dl (calcitonin and vehicle, respectively) in the 14 h after lights on trial. This result indicates that the serum total, as

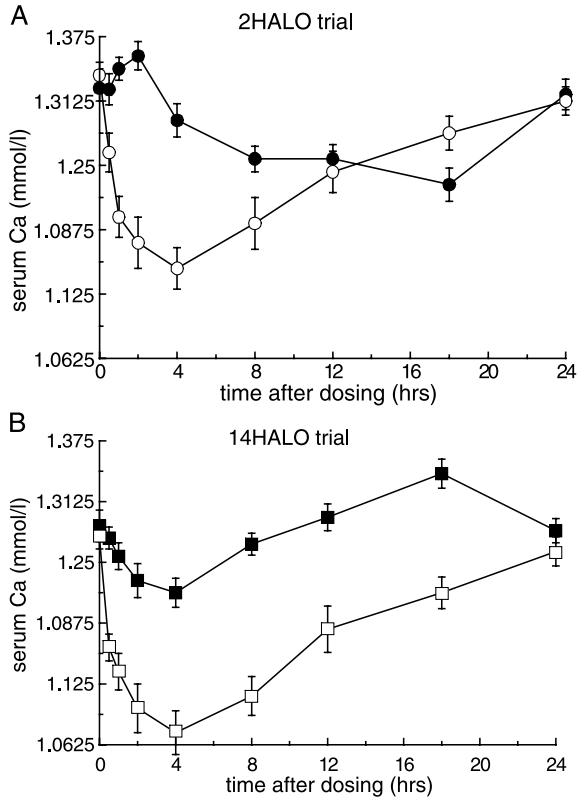

Fig. 1. Serum Ca concentration following a single injection of calcitonin at 2 'hour after light on' (HALO) (A) and 14 HALO (B). Mean ± S.E., $n = 10$ in each. Open circle and rectangle: calcitonin; closed circle and rectangle: vehicle.

well as ionized Ca concentration, was actually decreased by the drug. We also confirmed the diurnal change in serum total protein concentration in the vehicle group, which was reported previously (Tsuruoka et al., 2001; Wong et al., 1983). To evaluate the mechanism of the chronopharmacological effect of calcitonin, the urinary excretion of Ca and deoxypyridinoline was measured after a single dosing of calcitonin. As shown in Fig. 2, urine Ca/urine creatinine was not changed by the drug in the 2 and 14 h after lights on trials, although the basal value was significantly lower in the 14 h after lights on trial. Urinary deoxypyridinoline significantly ($P < 0.05$) decreased only in the 14 h after lights on trial (Fig. 2). We also measured serum salmon calcitonin concentration. However, no significant difference was observed in serum calcitonin at any points between the 2 and 14 h after lights on trials (data not shown). The area under the concentration curve was 264.3 ± 10.2 and 271.8 ± 12.5 pg h/ml (2 and 14 h after lights on, respectively). The apparent half-life of the hormone was 4.3 ± 0.5 and 5.1 ± 0.4 h (2 and 14 h after lights on, respectively).

3.2. Repeated-dosing study

Serum Ca concentration significantly increased after the repeated injection of vitamin D_3 (Fig. 3). After the injection of calcitonin, serum Ca gradually decreased in the 2 and 14 h after lights on trials, and the decrease was significantly ($P < 0.05$) greater in the 14 h after lights on group at 7 days after dosing. Serum total protein concentration was measured at the same times as the serum Ca, and it was not influenced by the injection of calcitonin (data not shown). The areas under the concentration curve of serum protein from 0 to 7 days were 53.8 ± 2.4 and 54.4 ± 2.9 mg day/dl (calcitonin and vehicle, respectively) in the 2 h after lights on trial and 52.7 ± 3.1 and 51.9 ± 2.7 mg h/dl (calcitonin and vehicle, respectively) in the 14 h after lights on trial. This result indicates that the serum ionized Ca^{2+} concentration also showed a dosing time-dependent reduction during calcitonin therapy. Urinary Ca excretion was measured after the final dose of calcitonin. Although the value

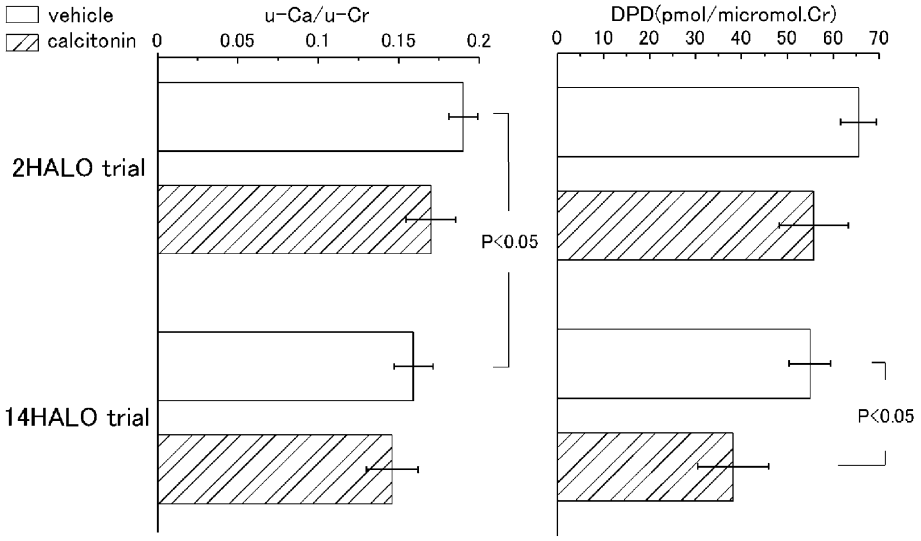

Fig. 2. Ratio of urinary Ca to creatinine (left panel) and deoxypyridinoline excretion (right panel) following a single injection of calcitonin at 2 and 14 HALO. Mean ± S.E., $n = 10$ in each. Open rectangle: vehicle; hatched rectangle: calcitonin.

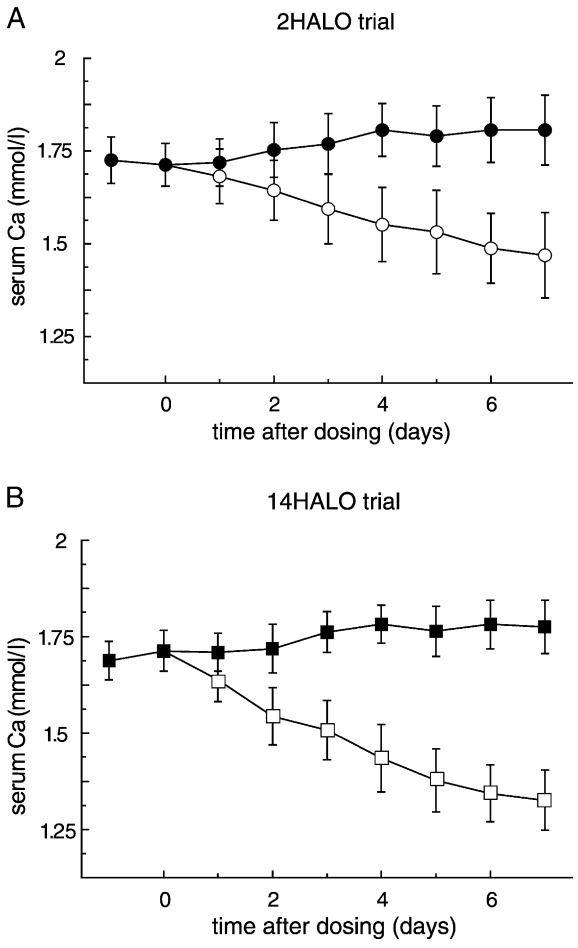

Fig. 3. Serum Ca concentration following a repeated injection of calcitonin at 2 HALO (A) and 14 HALO (B). Mean ± S.E., $n = 10$ in each. Open circle and rectangle: calcitonin; closed circle and rectangle: vehicle.

after vehicle treatment was significantly lower in the 14 h after lights on trial, calcitonin did not significantly affect the value in the 2 and 14 h after lights on trials (Fig. 4). Urinary deoxypyridinoline excretion after vehicle treatment was not different between the two trials (Fig. 4). Calcitonin significantly reduced deoxypyridinoline excretion in the 14 h after lights on trial, but not in the 2 h after lights on trial.

4. Discussion

In this single-dosing study, we found that the hypocalcemic effect of calcitonin was greater in the 14 h after lights on trial than in the 2 h after lights on trial in normal rats. We further observed that this effect persisted under repeated dosing of the drug in rats with vitamin D-induced hypercalcemia. To our knowledge, this is the first study to show that the therapeutic effect of calcitonin depends on the dosing time during repeated treatment. Calcitonin decreased the urinary excretion of cross-laps, the marker of bone resorption, in rats (Schlemmer et al., 1997), which indicates that calcitonin reduces bone resorption. Although calcitonin is reported to affect renal Ca handling, this effect varies with animal models and species (Quamme, 1980; Carney and Thompson, 1998). To evaluate the potential mechanism of the chronopharmacological effect of calcitonin, we collected urine in the single- and repeated-dosing studies. As shown in Fig. 3A and B, renal Ca excretion might not be involved in the mechanism of the dosing time-dependent hypocalcemic effect of calcitonin. However, this study showed that the dosing time-dependent change in bone resorption contributes to the effect. We did not detect a dosing time-dependent difference in extrinsic salmon calcitonin concentration in this study. Thus, we think that the sensitivity to calcitonin of

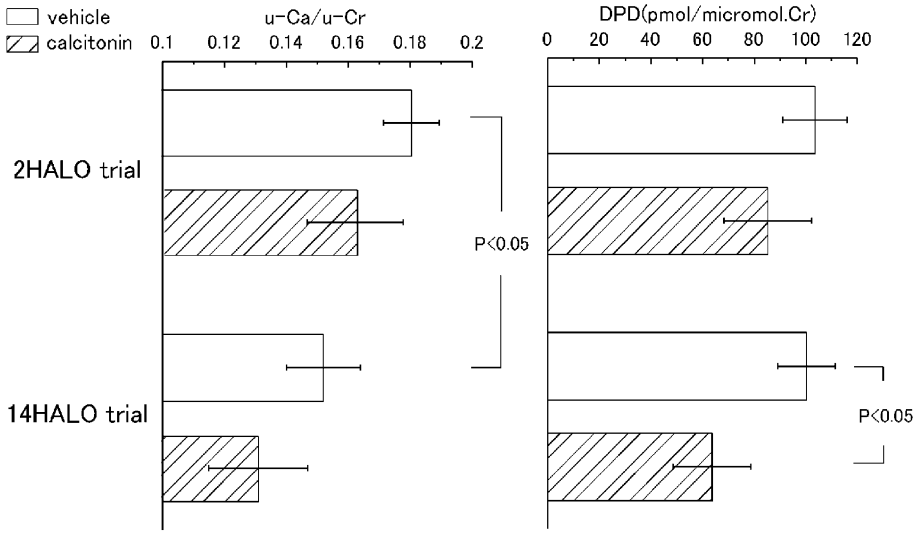

Fig. 4. Ratio of urinary Ca to creatinine (left panel) and deoxypyridinoline excretion (right panel) following repeated injection of calcitonin. Mean ± S.E., $n = 10$ in each. Open rectangle: vehicle; hatched rectangle: calcitonin.

osteoclasts varies with the calcitonin dosing time. It is reported that the intrinsic serum calcitonin concentration has a diurnal rhythm, with a peak during the late light phase and the early dark phase in rat (Lausson et al., 1985; Hirsch and Hagaman, 1982). Although the intrinsic hormone level is smaller than the extrinsic calcitonin concentration, the diurnal change may also contribute to this chronopharmacological phenomenon.

It is reported that the nasal absorption of salmon calcitonin depends on its dosing time in humans (Tarquini et al., 1988). It is probable that not only serum calcitonin concentration, but also the hypocalcemic effect, will vary with its dosing time after nasal administration. Because the nasal route is popular for calcitonin, these chronopharmacological profiles may be taken into consideration in clinical practice.

We confirmed a rapid reduction of serum Ca concentration at 4 h after injection of the vehicle in the 14 h after lights on group (Fig. 1B). Because we injected vehicle, it is possible that dilution of the blood by the vehicle may have caused the change. However, we think this is not the case because the reduction was not observed in the vehicle-injected control rats of the 2 h after lights on group (Fig. 1A). The volume of vehicle was rather small, and the finding that serum Ca concentration decreased at around 20 h after lights on is consistent with our previous finding.

In conclusion, we report that the hypocalcemic effect of calcitonin was greater when calcitonin was administered in the early dark phase. This dosing time-dependent effect was consistently observed in a single-dosing study with normal rats and in a repeated-dosing study with hypercalcemic rats. A dosing time-dependent reduction in bone reabsorption might be involved in the mechanism of this phenomenon. These results might be helpful in establishing an effective dosage regimen for calcitonin.

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