A comparison between daily and thrice-weekly i.v. administration of 1,25-dihydroxy-22-oxavitamin D₃ regarding suppression of parathyroid hormone secretion and calcaemic action in uraemic rats

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Abstract

Background. 1,25-Dihydroxy-22-oxavitamin D₃ (22-oxacalcitriol, OCT) is an analogue of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃, calcitriol) with less calcaemic activity, thus more suitable than 1,25(OH)₂D₃ for the control of parathyroid hormone (PTH) secretion in chronic dialysis patients. As the low-calcaemic action of OCT has been mainly attributed to its short half-life in the blood stream, the number of doses per week is the key factor to effective OCT therapy toward suppression of PTH secretion and hypercalcaemia. Thus, we investigated a comparison between daily and thrice-weekly i.v. administration of OCT regarding suppression of PTH secretion and calcaemic action in 5/6 nephrectomized rats as a model for chronic renal failure.

Methods. Model rats of chronic renal failure were made by 5/6 nephrectomy. At 3 months after surgery, they were administered either vehicle or OCT intravenously, daily (0.125 or 0.625 µg/kg) or thrice-weekly (0.6 or 3.0 µg/kg) for 2 weeks.

Results. The data show that 0.625 µg/kg/day (= 4.375 µg/kg/week) suppresses PTH secretion with significant increase in calcium levels at 24 h after the final administration, on the other hand, 3.0 µg/kg/thrice-weekly (= 9.0 µg/kg/week) suppresses PTH secretion, although moderate compared with 0.625 µg/kg/day, with a slight (not significant) increase in calcium.

Conclusions. The current clinical mode of OCT therapy, i.v. thrice-weekly administration, is a practically recommendable protocol.

Keywords: calcaemic action; OCT; parathyroid hormone; secondary hyperparathyroidism; uraemia; vitamin D analogue

Introduction

Patients with chronic renal failure frequently develop high-turnover bone disease due to excess parathyroid hormone (PTH) [1]. One of the principal mechanisms leading to secondary hyperparathyroidism (2HPT) is the reduced activation of vitamin D in the kidney. Thus, active vitamin D sterols such as 1α-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃, calcitriol) have been routinely used for the treatment of 2HPT associated with chronic renal failure. In addition, intermittent administration of supraphysiological doses of 1,25(OH)₂D₃ has been also introduced as treatment in the most severe cases [2,3]. Despite its effectiveness, this 1,25(OH)₂D₃ pulse therapy must sometimes be discontinued due to the development of hypercalcaemia. Development of another potential analogue of 1,25(OH)₂D₃ with a low-calcaemic activity is, therefore, highly desired.

1,25-Dihydroxy-22-oxavitamin D₃ (22-oxacalcitriol, OCT) is a synthetic analogue of vitamin D which was initially developed to mimic certain actions of 1,25(OH)₂D₃ on cell differentiation [4]. This analogue was soon recognized as a low-calcaemic agent for the treatment of hyperparathyroidism (HPT). In addition to the first report by Brown et al. [5] in normal rats, the usefulness of this analogue in the management of HPT in chronic renal failure was confirmed by other groups, using uraemic rats [6,7]. The subsequent comparable experiments between OCT and 1,25(OH)₂D₃ concerning the prevention of decrease of vitamin D receptor
content in parathyroid glands of uraemic rats [8] and the up-regulation of calcium-binding proteins in intestinal mucosa and kidney of uraemic rats [9], have clarified some biological characteristics of OCT disparate from 1,25(OH)2D3.

The preliminary results of clinical trials with OCT in chronic dialysis patients have already been reported [10]. In these dialysis patients, OCT is administered intravenously thrice-weekly at the end of each dialysis, which is identical to the mode of administration in 1,25(OH)2D3 pulse therapy. As the low-calcemic action of OCT has been attributed mainly to its short plasma half-life [11,12], dosing times per week are a key factor for OCT therapy toward suppression of PTH secretion and hypercalcemia. Thus, the dosing interval results obtained in uraemic rat experiments may bring important information for the clinical usage of OCT. In this study, we made a comparison between daily and thrice-weekly i.v. administration of OCT regarding suppression of PTH secretion and calcemic action in 5/6 nephrectomized rats as a model of chronic renal failure.

Materials and methods

Model of chronic renal failure

Six-week-old male Sprague–Dawley rats were purchased from S.L.C. Japan Co., Ltd (Tokyo, Japan). Rats with chronic renal failure were prepared by 5/6 nephrectomy with a standard two-step operation. After an acclimatization period of a week, the rats were anaesthetized by diethyl ether and two-thirds of the left kidney was surgically removed. One week later, a right nephrectomy was performed. In sham-operated rats, both kidneys were decapsulated. These rats were then maintained in sterilized cages and fed standard rodent chow containing 1.25% calcium and 1.06% phosphate (CE-2, Clea Japan Inc., Tokyo, Japan). Food and water were provided ad libitum. The present study was carried out in accordance with Chugai Pharmaceutical’s ethical guidelines of animal care, and the research protocols were approved by the animal care committee of the institution.

Administration of OCT

OCT was synthesized at Chugai Pharmaceutical Co., Ltd, Tokyo, Japan. It was dissolved in phosphate-buffered saline (pH 8.0) containing 0.2% ethanol and 0.01% Tween 20.

Three months after 5/6 nephrectomy, the uraemic rats were divided into five groups with equal levels of serum urea nitrogen (BUN), creatinine (CRE), and N-terminal PTH. These uraemic rats were given either vehicle solution (n = 7), OCT at 0.125 μg/kg (n = 4), and at 0.625 μg/kg (n = 4) daily for 2 weeks, or OCT 0.6 μg/kg (n = 4) and 3.0 μg/kg (n = 4) thrice-weekly for 2 weeks into the tail vein. We selected these doses because our previous study demonstrated that i.p. administration of OCT at 0.125 μg/kg daily for 2 weeks leads to marked hypercalcemia with suppressed PTH levels (data not shown). Sham-operated rats were given vehicle for only 2 weeks. The volume of the vehicle for single injection was 0.1 ml/100 g body weight.

At 24 h after the final injection, rats were killed under diethyl ether anaesthesia and blood samples were obtained from the abdominal aorta.

Serum biochemistry

BUN and CRE were measured by COBAS FARAII (Roche Diagnostica, Tokyo, Japan). Total serum calcium (Ca) and phosphorous (P) were determined using Ca C-test WAKO (Wako Junyaku, Tokyo, Japan) and Pi SET (latron, Tokyo, Japan). Immunoreactive serum N-terminal PTH was measured by a rat PTH (IRMA) kit (Immutopics, Inc., CA, USA).

Statistical analysis

All data are expressed as means ± standard error. Statistical analysis was performed with SAS system. Dunnet’s method was used for the comparison of vehicle-treated uraemic group with OCT-treated groups and unpaired t-test was used for the comparison between sham-operated group and vehicle-treated uraemic group. P values <0.05 was considered significant.

Results

Body weight and biochemical parameters measured at the time of introduction of OCT treatment (3 months after 5/6 nephrectomy) and at the time of death (24 h after the final administration) are shown in Table 1. There was no significant difference in body weight in all groups including sham and uraemic groups. After 3.5 months of renal failure, serum CRE and BUN increased in the vehicle-treated group (1.37 ± 0.09 and 60.0 ± 5.3 mg/dl, respectively), compared with the sham-operated group (0.79 ± 0.04 and 17.1 ± 0.5 mg/dl, P < 0.05, respectively). Serum CRE and BUN in the OCT-treated groups were not significantly different from the vehicle-treated group.

Compared with the vehicle-treated group rats, serum Ca was significantly increased in uraemic rats receiving the 0.625 μg/kg/daily dose of OCT but the increase was not significant in those receiving the 3.0 μg/kg/thrice-weekly dose (10.7 ± 0.4 vs 9.2 ± 0.2 mg/dl, P < 0.05 and 10.0 ± 0.1 vs 9.2 ± 0.2 mg/dl, not significant (NS), respectively). The direct comparison between the 0.625 μg/kg/daily group and the 3.0 μg/kg/thrice-weekly group did not show a significant difference (10.7 ± 0.4 vs 10.0 ± 0.1 mg/dl, NS). Neither doses of OCT (0.125 μg/kg/daily and 0.6 μg/kg/thrice-weekly) caused a significant increase in Ca.

Serum P was increased in the vehicle-treated group (6.3 ± 0.5 mg/dl) and in groups receiving 0.625 μg/kg/daily and 3.0 μg/kg/thrice-weekly doses of OCT (7.4 ± 1.2 and 8.1 ± 1.3 mg/dl, respectively), compared with the sham-operated group (5.1 ± 0.1 mg/dl), although the difference was not significant.

Serum N-terminal PTH (Figure 1) increased from 12.7 ± 1.0 pg/ml in the sham-operated group rats to 75.2 ± 22.5 pg/ml in the vehicle-treated group rats...
demonstrating the development of 2HPT ($P < 0.05$). Both the $0.625 \mu g/kg$ daily and $3.0 \mu g/kg$ thrice-weekly doses of OCT significantly blocked the increase in levels of N-terminal PTH ($5.3 \pm 1.5 vs 75.2 \pm 22.5 \text{pg/ml}$, $P < 0.001$, $15.1 \pm 3.8 vs 75.2 \pm 22.5 \text{pg/ml}$, $P < 0.05$, $5.3 \pm 1.5 vs 15.1 \pm 3.8 \text{pg/ml}$, NS, respectively). The $3.0 \mu g/kg$ (thrice-weekly dose of OCT, however, produced this effect without significant increase of Ca. The data show that $0.625 \mu g/kg$ (daily and $3.0 \mu g/kg$ (thrice-weekly) suppresses PTH secretion with significant increase in serum Ca levels at 24 h after the final administration. On the other hand, $3.0 \mu g/kg$ (thrice-weekly) ($= 9.0 \mu g/kg/week$) suppresses PTH secretion, although moderate compared with $0.625 \mu g/kg/day$, with a slight (not significant) increase in Ca levels.

**Discussion**

High-turnover bone disease due to excess PTH is the main feature of renal osteodystrophy in chronic dialysis patients [1]. Treatment of this abnormality aims at ameliorating several stimuli for PTH secretion such as hypocalcaemia, hyperphosphataemia, and decreased activation of vitamin D. Thus, phosphate binders and active vitamin D sterols have been routinely used. In addition, resistance of parathyroid cells to $1,25(OH)_2D_3$ due to decreased density of $1,25(OH)_2D_3$ receptors is recognized as a cause of parathyroid hyperfunction in chronic renal failure [13]. Accordingly, supraphysiological doses of $1,25(OH)_2D_3$ by intermittent i.v. [2] or oral [3] administration (pulse therapy) have become popular as treatment for severe HPT in chronic renal failure. However, very high doses of $1,25(OH)_2D_3$ are needed to suppress PTH secretion in the most severe cases. High doses of $1,25(OH)_2D_3$ often causes hypercalcaemia, for which this therapy should be discontinued. It is, therefore, necessary to develop a new vitamin D analogue which shows stronger and more selective effects on parathyroid than those of $1,25(OH)_2D_3$.

OCT is a new synthetic analogue of $1,25(OH)_2D_3$ with various potent biological activities. These include among others induction of cell differentiation [4], and immunoregulating effects in mice without inducing hypercalcaemia. Such a low-calcemic action of OCT soon attracted the attention of the nephrological community. Accordingly, Brown et al. [5] demonstrated that OCT suppressed PTH secretion

**Fig. 1.** Effects of OCT on serum N-terminal PTH levels after daily (0.125 and 0.625 $\mu g/kg$) and thrice-weekly (0.6 and 3.0 $\mu g/kg$) administration. $^aP < 0.05$ vs sham-operated group. $^*P < 0.05$ vs vehicle-treated group.

**Table 1.** The effects of OCT on biochemical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham</th>
<th>Vehicle</th>
<th>Daily ($\mu g/kg$)</th>
<th>Thrice-weekly ($\mu g/kg$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.125</td>
<td>0.625</td>
<td>0.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>485.2 ± 12.4</td>
<td>455.7 ± 9.0</td>
<td>445.5 ± 21.9</td>
<td>464.5 ± 13.5</td>
</tr>
<tr>
<td>Ca, mg/dl</td>
<td>8.8 ± 0.1</td>
<td>9.2 ± 0.2</td>
<td>9.8 ± 0.3</td>
<td>10.7 ± 0.4 $^a$</td>
</tr>
<tr>
<td>Pi, mg/dl</td>
<td>5.1 ± 0.1</td>
<td>6.3 ± 0.5</td>
<td>7.9 ± 1.6</td>
<td>7.4 ± 1.2</td>
</tr>
<tr>
<td>CRE, mg/dl</td>
<td>0.79 ± 0.04</td>
<td>1.37 ± 0.09 $^a$</td>
<td>1.85 ± 0.36</td>
<td>1.77 ± 0.31</td>
</tr>
<tr>
<td>Bun, mg/dl</td>
<td>17.1 ± 0.5</td>
<td>60.0 ± 5.3 $^a$</td>
<td>64.3 ± 18.1</td>
<td>71.0 ± 11.9</td>
</tr>
</tbody>
</table>

$^aP < 0.05$ vs sham-operated group.

$^bP < 0.05$ vs vehicle-treated group.
and synthesis in normal rats. The efficacy of OCT was also confirmed in uraemic rats by several groups [6,7] and some disparate biological characters of OCT from 1,25(OH)2D3 were investigated by us and other groups [8,9,12].

As presented in the present study, it was confirmed that the thrice-weekly administration of OCT suppresses PTH secretion effectively as well as daily treatment. We further demonstrated that higher doses of OCT thrice-weekly could be administered with less risk of calcaemic action than by daily administration. Although we did not investigate a precise time-course change of calcium levels after OCT treatment by thrice-weekly and daily administration in the present study, the available data show that up-regulated calbindin D9k mRNA levels by OCT return to pretreatment levels rapidly [12]. We presume that the increase of serum calcium concentration by OCT, if any, was transient. The most important issue of thrice-weekly administration of OCT is the normalization of increased calcium levels before the next treatment. A potential explanation of such a characteristic of OCT may be related to its pharmacokinetics [11,12]. As OCT has a lower affinity for the vitamin D-binding protein than 1,25(OH)2D3, unbound OCT would be more susceptible to degradation and would be cleared more rapidly from the circulation. Actually, the plasma half-life of OCT in uraemic dogs was reported to be 2.1 ± 0.2 h compared with 9–10 h for 1,25(OH)2D3 [14]. The calcaemic effect of OCT might, therefore, be further decreased by thrice-weekly administration with an interval, rather than by daily administration. On the other hand, as shown with 1,25(OH)2D3 administration by Reichel et al. [15], the peak level is more important than the total dose administered, not only for the suppression of PTH, but also for the suppression of parathyroid hyperplasia. Thus, large dose/thrice-weekly administration should be able to achieve a much higher peak concentration than small dose/daily administration.

Taking these mechanisms into consideration, thrice-weekly administration of OCT might possibly suppress parathyroid function with less risk of hypercalcaemia than daily administration of OCT. Thus, the current clinical mode of OCT therapy, i.v. thrice-weekly administration, is a practically recommendable protocol. Further quantitative comparative studies between OCT and 1,25(OH)2D3 with intermittent administration regarding suppression of PTH secretion and calcaemic action in uraemic rats are reported in the next article of this Supplement.

References