Effects of 1α-Hydroxyvitamin D₃ on Experimental Uremic Renal Osteodystrophy in Rats Induced by Na-Sulfacetethylthiazole*

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Synopsis

Na-sulfacetethylthiazole (SAT), 0.1 g/kg body weight as 5% aqueous solution, was injected intraperitoneally to Wistar male rats weighing 200 to 300 g, twice a week for 1, 2 and 4 months, while 1α-hydroxyvitamin D₃ (1α-OH-D₃) was simultaneously administered orally to a half of the SAT-4 month-treated rats at a daily dose of 0.25 μg/kg body weight for the last 19 days of the feeding period. Both blood urea nitrogen (BUN) and serum inorganic phosphorus concentrations were markedly increased and the histological examination of the kidneys of SAT-treated rats revealed interstitial nephritis. Serum calcium level was significantly decreased in the rats treated with SAT for 2 or 4 months. Serum parathyroid hormone (PTH) level as well as the wet weight of parathyroid glands was increased in SAT-treated rats, while simultaneous administration of 1α-OH-D₃ inhibited such increases. Serum 25-hydroxyvitamin D₃ (25-OH-D₃) was decreased in the rats treated with SAT for 2 months. The X-ray density and calcium content of the femurs of SAT-treated rats were decreased, while simultaneous administration of 1α-OH-D₃ inhibited such decreases. Tetrachrome stain of the femurs of SAT-4 month-treated rats revealed a marked increase of osteoid contents in the bone cortex, while 1α-OH-D₃ inhibited such an increase in osteoid formation. These data indicate that 1α-OH-D₃ would be effective for the treatment of uremic renal osteodystrophy, although its detailed mechanism remains to be further clarified.

Although various methods have been reported for the preparation of experimental renal failure, there have been only a few in which they induced uremic renal osteodystrophy. Egar (1949) used platinum, copper, lead or uranium for the production of renal failure leading to parathyroid hyperplasia and osteitis fibrosa. Okano et al. (1972 and 1974) already reported increased contents of parathyroid hormone (PTH) in the parathyroid glands as well as bone changes in the rat with SAT-induced acute renal failure. Nishii et al. (1978) recently reported the bone lesions in the rats by inducing chronic glomerulonephritis through the administration of a glycopeptide. In the present study, subchronic renal failure was induced in rats by administration of a smaller dose of SAT for up to 4 months in order to further elucidate the mechanism of abnormal calcium metabolism as well as uremic renal osteodystrophy and to evaluate the therapeutic effect of 1α-OH-D₃ on those changes.

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Materials and Methods

Male Wistar rats weighing 200 to 300 g were maintained on Oriental Rat Chow (calcium 1.85%, inorganic phosphorus 1.04%, vitamin D3 200 IU/g) and tap water ad libitum. SAT, 0.1 g/kg body weight was injected intraperitoneally to rats as 5% aqueous solution twice a week for 1, 2 and 4 months. To a half of SAT-4 month-treated group, 0.25 μg/kg body weight of 1α-OH-D3 dissolved in O.D.O. was orally administered for the last 19 days of the experimental period. These rats were bled by cutting the jugular vein under ether anesthesia followed by removing parathyroid glands, kidneys and femurs. X-ray films of the femurs were taken by soft X-ray (Softex) apparatus along with a stepshaped aluminium plate as standard. The X-ray density of the femur was determined by a densitometer and expressed as the corresponding thickness of aluminium in mm. The femurs and kidneys were dried at 80°C overnight, incinerated through heating at 800°C for 12 hr and dissolved in 6 N HCl solution for the determination of calcium content.

Rat serum PTH was determined by modifying the radioimmunoassay method reported by Okano et al. (1974). Highly purified bovine 1-84 PTH (Wilson Co., Ltd.) was labelled with 125I by the chloramine-T method followed by purification through BioGel P-10 column chromatography. Antiserum specific for both C- and N-terminal region of bovine PTH was produced by injecting bovine PTH trichloroacetic acid (TCA)-powder to guinea pigs. Mixture of 100 μl of antibovine PTH guinea pig serum (final dilution 1:100,000), 100 μl of standard 1-84 bovine PTH diluted serially with Quso G32-treated serum or sample, 200 μl of 0.05 M Veronal buffer (pH 8.6) containing 1% Quso G32-treated serum and 0.05% thimerosal was incubated at 4°C for 4 days. Then, 100 μl of 125I-PTH was added. After further 2-day incubation, 100 μl of appropriately diluted goat anti-guinea pig γ-globulin (Antibodies Incorporated) was added for the separation of bound and free of PTH. A standard curve of bovine PTH was parallel to a dilution curve of SAT-treated rat serum, and 10% fall from 0 standard was 0.10 ng/ml (Fig. 1). Rat serum 25-OH-D3 was determined by competitive protein binding assay as already reported (Okano et al., 1976). In this assay, cross reaction with vitamin D2, 1, 25(OH)2D3 and 1α-OH-D3 was 2.18%, 0.7% and less than 0.5%, respectively.

The femurs were fixed in 70% ethanol for tetra-chrome stain (Villanueva et al., 1964). Kidneys were fixed in 60% formalin for Hematoxylin and Eosin stain. BUN was measured according to the indo-phenol autoanalyzer method, serum calcium according to orthocresolphthalein complexon (OCPC)-autoanalyzer method and serum inorganic phosphorus according to the molybdenum disulfide method.

![Fig. 1. Dilution curves of bovine 1-84 PTH (bPTH) and SAT-treated rat serum. The solid line indicates 1-84 bPTH, the dotted line indicates SAT-treated rat serum.](image-url)
Results

Pathological findings of the kidneys of SAT- and 1α-OH-D₃-treated rats.

The kidneys of the rats treated with SAT for 1 and 2 months were markedly swollen and the surface was finely granular, turbid and yellowish. The kidneys of SAT-4 month-treated rats were rather atrophic and whitish small materials were observed on the surface which were identified as calcified substances by Softex films. H-E stain of the kidneys of SAT-treated rats revealed the findings of interstitial nephritis with marked round cell infiltration into the interstitial space, necrosis and calcification of the tubular epithelium, and obstruction of the tubules with SAT crystals and casts of blood cells.

Effects of SAT and 1α-OH-D₃ on rat BUN, serum inorganic phosphorus and serum calcium

As shown in Table 1, BUN was significantly increased in all of the SAT-treated groups compared to that of the control group, while serum inorganic phosphorus was significantly increased in the SAT-1 month-treated group, SAT-4 month-treated group and SAT-4 month plus 1α-OH-D₃-treated group. As shown in Fig. 2, serum calcium levels of SAT-treated rats were significantly decreased in the SAT-2 month-treated group and the SAT-4 month-treated group compared to that of control group, while in SAT-4 month plus 1α-OH-D₃-treated rats, it tended to be increased in 3 of 4 animals.

Effects of SAT and 1α-OH-D₃ on wet weight and histological findings of rat parathyroid glands.

As shown in Table 1, wet weight of parathyroid glands was increased in SAT-treated rats, while it was decreased in the SAT-treated rats simultaneously admini-

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>BUN (mg/100 ml)</th>
<th>Serum inorganic phosphorus (mg/100 ml)</th>
<th>Parathyroid gland (mg. wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.2 ± 1.3±</td>
<td>7.3 ± 0.1</td>
<td>0.78 ± 0.05</td>
</tr>
<tr>
<td>SAT 1 month</td>
<td>89.2 ± 12.2***</td>
<td>10.5 ± 0.2***</td>
<td>3.0 ± 0.7*</td>
</tr>
<tr>
<td>SAT 2 month</td>
<td>102.0 ± 25.1***</td>
<td>9.3 ± 1.2</td>
<td>11.9 ± 3.0**</td>
</tr>
<tr>
<td>SAT 4 month</td>
<td>71.7 ± 5.8***</td>
<td>9.5 ± 0.4***</td>
<td>13.2 ± 2.5***</td>
</tr>
<tr>
<td>SAT 4 month + 1α-OH-D₃</td>
<td>119.0 ± 17.6***</td>
<td>14.2 ± 2.2*</td>
<td>4.6 ± 1.6</td>
</tr>
</tbody>
</table>

SAT, 0.1g/kg body weight, was injected intraperitoneally twice a week.
1α-OH-D₃, 0.25 µg/kg body weight, was orally administered for the last 19 days of the experimental period.

† Mean±S.E.
The number of rats is shown in parentheses.
Difference from control: * P<0.02, ** P<0.01, *** P<0.001.
tered with 1α-OH-D₃. H-E stain of the parathyroid glands of SAT-treated rats revealed marked hyperplasia of chief cells.

**Effects of SAT and 1α-OH-D₃ on rat serum PTH and 25-OH-D₃ levels.**

As shown in Fig. 3, serum PTH levels of control rats were 0.10 ng/ml or below. Average PTH levels of the rats treated with SAT for 1, 2 and 4 months, were 1.13 ng/ml, 0.64 ng/ml, and 0.61 ng/ml, respectively. On the other hand, simultaneous administration of 1α-OH-D₃ in the SAT-4 month-treated group decreased PTH levels in 3 of 4 animals. As shown in Fig. 4, serum 25-OH-D₃ levels were significantly decreased in the SAT-2 month-treated group.

**Effects of SAT and 1α-OH-D₃ on X-ray density and calcium content of rat femur.**

As shown in Table 2, the X-ray density of rat femurs was significantly decreased in the groups treated with SAT for 1, 2 and 4 months. However, simultaneous administration of 1α-OH-D₃ to SAT-4 month-treated rats inhibited such a decrease. As shown in Table 2, the calcium content of rat femurs was significantly decreased in SAT-2 month-treated group and SAT-4 month-treated group.

![Fig. 3. Effects of SAT and 1α-OH-D₃ on the serum PTH level in rats.](image)

![Fig. 4. Effects of SAT and 1α-OH-D₃ on the serum 25-OH-D₃ level in rats. Vertical bars indicate S.E. * and ** P<0.01.](image)

**Table 2. Effects of SAT and 1α-OH-D₃ on the X-ray density and calcium content of rat femurs.**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>X-ray density (mm, aluminium)</th>
<th>Calcium content (% dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(5)</td>
<td>0.72±0.02</td>
</tr>
<tr>
<td>SAT 1 month</td>
<td>(4)</td>
<td>0.60±0.01***</td>
</tr>
<tr>
<td>SAT 2 month</td>
<td>(5)</td>
<td>0.57±0.02*</td>
</tr>
<tr>
<td>SAT 4 month</td>
<td>(4)</td>
<td>0.63±0.02*</td>
</tr>
<tr>
<td>SAT 4 month+1α-OH-D₃</td>
<td>(4)</td>
<td>0.76±0.02*</td>
</tr>
</tbody>
</table>

SAT, 0.1g/kg body weight, was injected intraperitoneally twice a week.

1α-OH-D₃, 0.25 μg/kg body weight, was orally administered for the last 19 days of the feeding period. The number of rats is shown in parentheses.

* Mean±S.E.

Difference from control: * P<0.05, ** P<0.02, *** P<0.01, † P<0.001.

* and ‡ P<0.001.
Pathological findings of the femurs of SAT- and 1α-OH-D₃-treated rats.
As shown in Fig. 5, tetrachrome stain of the femur of SAT-4 month-treated rats revealed the prominently increased formation of osteoid in the bone cortex, while simultaneous administration of 1α-OH-D₃ with SAT decreased such an increase of osteoid formation in the bone cortex.

Correlation among serum PTH, serum 25-OH-D₃ and serum calcium.
As shown in Fig. 6, significant negative correlation was observed between serum PTH and serum calcium in the SAT-treated group (P<0.01). As shown in Fig. 7, a significant positive correlation was observed between serum 25-OH-D₃ and serum calcium (P<0.05). However, no significant correlation was observed between serum PTH and serum 25-OH-D₃.

Discussion
Clinically, osteodystrophy is a well recognized complication of chronic renal failure varying from rickets or osteomalacia, to bone changes similar to osteoporosis and osteitis fibrosa. In experimental studies, however, there have been a few reports on...
the bone changes and abnormal calcium metabolism in chronic renal failure. Egar (1949) reported parathyroid hyperplasia and osteitis fibrosa in rats by inducing renal injury through the administration of platinum, copper, lead or uranium, and the prevention of such bone lesions by parathyroidectomy. Okano et al. (1972) reported increased contents of PTH in the parathyroid glands and bone changes similar to osteoporosis in the rats with acute renal failure induced by a short term administration of SAT. Mawer et al. (1973) reported the failure of formation of 1,25-dihydroxyvitamin D$_3$ in chronic renal failure. After this report, 1,25(OH)$_2$D$_3$ or its synthetic precursor, 1a-OH-D$_3$, has been tried for the treatment of renal osteodystrophy in man (Chalmers et al., 1973; Davie et al. 1976). Nishii et al. (1978) recently reported the bone lesions in the rats with chronic glomerulonephritis by the administration of a glycopeptide, along with the preventive effect of 1a-OH-D$_3$ on such bone lesions. In the present study, subchronic renal failure was induced by a longer term administration of a smaller dose of SAT to rats, and the therapeutic effect of 1a-OH-D$_3$ on the bone changes in these SAT-treated rats was investigated.

A decrease of serum 25-OH-D$_3$ levels in the rats with SAT-induced renal failure might be attributed to malnutrition regardless of pair feeding and to the possible urinary excretion of 25-OH-D$_3$ in accompany with proteinuria. On the other hand, because there is 10 to 30% cross reaction of 24, 25-dihydroxyvitamin D$_3$ (24, 25(OH)$_2$D$_3$) in the current competitive protein binding assay of 25-OH-D$_3$ (Haddad et al., 1976), it might be conceivable that a decrease of serum 24, 25(OH)$_2$D$_3$ level in the rats with renal failure would result in an apparent decrease of serum 25-OH-D$_3$ level in this assay, prompting us to establish an assay method of 24, 25 (OH)$_2$D$_3$. A positive correlation between serum 25-OH-D$_3$ level and serum calcium level in the present study proposes a possibility that both uptake of vitamin D$_3$ and calcium would be decreased in renal failure, and that a decrease in serum 25-OH-D$_3$ and/or 24, 25 (OH)$_2$D$_3$ would result in a decrease of serum calcium level, suggesting that 24, 25 (OH)$_2$D$_3$ might be concerned with intestinal calcium absorption and bone resorption.

Microradiograph of the femur of SAT-treated rats revealed an increased number of bone resorption cavities in the bone cortex, which is compatible with the findings of a moderate degree of hyperparathyroidism. Tetrachrome stain of the femur of SAT-4 month-treated rats revealed a marked increase of osteoid formation in the bone cortex, while the simultaneous administration of 1a-OH-D$_3$ with SAT treatment definitely decreased the amount of osteoid in the femur cortex, indicating a therapeutic effect of 1a-OH-D$_3$ on SAT-induced renal osteodystrophy. As to the mechanisms for such effects of 1a-OH-D$_3$ on uremic renal osteodystrophy as well as abnormal calcium metabolism such as the increased serum PTH level and decreased serum calcium level, it is presumable that 1a, 25(OH)$_2$D$_3$ converted from 1a-OH-D$_3$ in the liver promotes intestinal calcium absorption and calcification of osteoid, and that an increased level of serum calcium decreases PTH secretion, while a direct inhibition of PTH secretion by 1a, 25(OH)$_2$D$_3$ has also to be considered (Chertow et al., 1975). The physiological significance of 24, 25 (OH)$_2$D$_3$ still remains unclarified, although it was reported that it inhibited PTH secretion (Care et al., 1975; Henry et al., 1976) and promoted calcification of osteoid tissues.
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References


