Effect of Parathyroidectomy on Bone Atrophy Induced by Renal Injury with Na-Sulfacetylthiazole

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Synopsis

Intraperitoneal administration of 0.1 to 0.2 gm/kg Na-Sulfacetylthiazole (SAT) twice a week for 4 weeks induced obstructive nephropathy, and bone changes similar to osteoporosis with a decrease in the X-ray density and calcium content of the tail bone and femur, decrease of femoral cortical thickness and an increase in bone resorption cavities in 3 month old rats. Such bone changes, prevented through prior parathyroidectomy, might be due to increased parathyroid hormone activity secondary to SAT-induced renal injury.

Na-Sulfacetylthiazole (SAT) is known to induce obstructive nephropathy with the histological findings of interstitial nephritis, medionecrosis with calcification of the aorta, and myocarditis with myocardial necrosis and calcification in rats (Lehr, 1959; Okano et al., 1970). In the pathogenesis of such cardiovascular changes, increased parathyroid hormone activity secondary to SAT-induced renal failure has been regarded as important, in view of the preventive effect of prior parathyroidectomy (Lehr, 1959; Okano et al., 1970). Okano et al., (1970) reported mobilization of Ca from the stable bone fraction to the soft tissues after single injection of SAT, suggesting the role of parathyroid hormone in stimulating bone resorption. However, scarcely any attention has been directed to bone changes in animals with renal injury caused by SAT treatment. In the present study, bone changes were quantitatively studied with reference to administration of SAT, by means of measurement of calcium content, roentgenography and microradiography.

Materials and Methods

Forty-eight male rats of Wistar strain at the age of 3 months weighing 250 to 350 g were divided into six equal groups and pair-fed on Oriental Rat Chow (Ca 1.76%, P 1.04%, VD 200 IU/100 g) and tap water ad libitum. Parathyroidectomy was performed with hot wire cautery one week prior to the administration of SAT in 2 groups. In 3 groups of intact rats, SAT, 0.2 g, 0.1 g or 0.05 g per kg body weight as 5% aqueous solution, was injected intraperitoneally twice a week for 4 weeks, and in one group of parathyroidectomized rats 0.2 g/kg SAT was similarly injected, while only distilled water was injected intraperitoneally in the remaining intact and parathyroidectomized rats serving as controls. After repeated injections of SAT for 4 weeks, animals were sacrificed by blood letting under ether anesthesia to obtain femora and tails as well as kindey, aorta and heart. Parathyroid glands were removed under a dissecting microscope. X-ray films of bones were taken with Softex soft X-ray apparatus (CMB type, F, target-specimen distance 45 cm, 30 V, 2.5 mA, 15 sec) along with stepshepared standard aluminium plates as standard. The X-ray density of the diaphyses of the 5th tail bones was determined by Aloka Thin-layer Chromatogram Scan.
The X-ray density of the middiaphyses of rat tail bones in soft X-ray film, was decreased in intact animals treated with 0.2 g/Kg or 0.1 g/Kg SAT twice a week for 4 weeks but not in parathyroidectomized rats treated with 0.2 g/Kg SAT similarly (Fig. 1, Table 1.) The calcium content of the rat tail bone was decreased in response to the administration of 0.2 g/Kg SAT for 4 weeks in intact rats, while prior parathyroidectomy prevented such a decrease (Table 1).

The bone density and cortical thickness of the rat femora in soft X-ray film were decreased after the administration of 0.2 g/Kg SAT twice a week for 4 weeks to intact rats, while prior parathyroidectomy prevented these changes (Fig. 2, Table 1). The calcium content of the rat femur was decreased after the administration of 0.2 g/Kg or 0.1 g/Kg SAT for 4 weeks in intact rats, while prior parathyroidectomy prevented such a decrease (Table 1).

Table 1. Effect of Na-sulfacetylthiazole (SAT) and parathyroidectomy (PTX) on the X-ray density, cortical thickness and calcium content of the 5th tail bone and femur of rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Tail bone (mm AL)</th>
<th>Ca (mg)/100 mg dry wt</th>
<th>Cortical thickness (%)</th>
<th>Ca (mg)/100 mg dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>1.12 ± 0.07*</td>
<td>18.2 ± 0.8§</td>
<td>25.0 ± 0.9*</td>
<td>28.4 ± 1.3†</td>
</tr>
<tr>
<td>SAT 0.2 g/kg ip (4)</td>
<td>0.69 ± 0.09**</td>
<td>15.5 ± 0.3§§</td>
<td>19.4 ± 1.6**</td>
<td>23.8 ± 1.6††</td>
</tr>
<tr>
<td>SAT 0.1 g/kg ip (8)</td>
<td>0.83 ± 0.06***</td>
<td>16.7 ± 0.8</td>
<td>20.5 ± 3.5</td>
<td>25.0 ± 0.8§</td>
</tr>
<tr>
<td>SAT 0.05 g/kg ip (8)</td>
<td>1.15 ± 0.09</td>
<td>19.6 ± 0.2</td>
<td>22.1 ± 1.5</td>
<td>28.1 ± 0.2</td>
</tr>
<tr>
<td>PTX + SAT 0.2 g/kg ip (4)</td>
<td>1.21 ± 0.02†</td>
<td>17.8 ± 0.4††</td>
<td>25.0 ± 3.2</td>
<td>28.6 ± 1.2</td>
</tr>
<tr>
<td>PTX (6)</td>
<td>1.25 ± 0.06</td>
<td>19.5 ± 0.4</td>
<td>25.9 ± 0.7</td>
<td>28.3 ± 1.1</td>
</tr>
</tbody>
</table>

SAT was injected intraperitoneally twice a week for 4 weeks.
Number of rats in parenthesis.
Mean ± SEM.
* and **; * and *** P < 0.01, ** and † P < 0.02, § and §§ P < 0.05, §§ and †† P < 0.01 (tail bone).
* and ** P < 0.02, † and ††; † and § P < 0.05 (femur).
Fig. 1. Soft X-ray picture of the rat tail bone. Softex (CMB type), target-specimen distance 45 cm, F, 30V, 2.5 mA, 15 sec. 1. Control. 2. After 4 weeks of treatment with 0.2 g/kg SAT twice a week intraperitoneally. 3. After similar treatment with 0.1 g/kg SAT. 4. After parathyroidectomy and 4 weeks of treatment with 0.2 g/kg SAT twice a week intraperitoneally. 5. After parathyroidectomy. Note the marked decrease in the density of tail bone after SAT treatment and the preventive effect of prior parathyroidectomy.
Fig. 2. Soft X-ray picture of the femur of the rats. Softex (CMB type), target-specimen distance 45 cm, F, 30V, 2.5 mA, 15 sec. SAT (0.2 g/kg or 0.1 g/kg) was intraperitoneally injected twice a week for 4 weeks. Note the decrease in the bone density and cortical thickness of the femur after SAT treatment and the preventive effect of prior parathyroidectomy.

Morphological findings of rat bone

Microradiogram of the entire cross section of the femur revealed an increase in the number of bone resorption cavities in the cortex of the femora of SAC-treated rats compared to the control animals (Fig. 3).

In the undecalcified cross sections of the rat femora which were stained by the method of von Kossa, the thickness of the osteoid seams showed no difference between SAC-treated rats and intact rats.

Cortical thickness of the diaphyses of the rat tail bone was decreased in intact rats treated with SAT. Trabeculae in the metaphyses of SAT-treated rats were thin and decreased in number. However, there was neither increase in the number of osteoclasts nor fibrotic changes of bone marrow in the tail bone of the SAT-treated rats.

Histological findings of the kidney, aorta and heart of SAT-treated rats

Kidneys of SAT-treated rats were swollen with fine granular, turbid and yellowish appearance of the surface, with histological findings of interstitial nephritis and nephrocalcinosis. Aortae of SAT-treated rats showed medionecrosis and medial calcification. Hearts of SAT-treated rats showed myocarditis with myocardial necrosis and calcification. Prior parathyroidectomy prevented such changes induced by SAT except for interstitial nephritis.

Serum calcium, inorganic phosphorus and blood urea nitrogen of the rat

Serum calcium level of the parathyroidectomized rats was definitely lower than that of intact rats. There was no significant difference between the serum calcium level of SAT-treated rats and that of control animals. Serum inorganic phosphorus and blood urea nitrogen was markedly increased after the treatment with SAT (Table 2).

Findings of the rat parathyroid glands

The area of the central section of the parathyroid glands fixed in 15% neutral formalin was $0.60 \pm 0.04 \, \text{mm}^2$ in SAT-treated rats ($n = 5$) and $0.34 \pm 0.07 \, \text{mm}^2$ in control rats ($n = 5$) ($\text{Mean} \pm \text{SE} \times, p < 0.02$). The ratio of cytoplasma and nuclei in the parathyroid glands was $3.5 \pm 0.21$ in SAT-treated rats ($n = 5$) and $2.7 \pm 0.18$ in control animals ($n = 5$) ($\text{Mean} \pm \text{SEM}, p < 0.02$).

Effect of SAT on the urinary excretion of inorganic phosphorus in intact and parathyroidectomized rats

Mean daily urinary excretion of inorganic
Fig. 3. Microradiogram of the cross section of the rat femur (×100).
Left: SAT (0.2 g/kg) was intraperitoneally injected twice a week for 4 weeks.
Right: Control. Note the increase in the number of bone resorption cavities in the bone cortex of SAT-treated rats.

Table 2. Effect of Na-sulfacetylthiazole (SAT) on serum Ca, serum P and BUN of intact and parathyroidectomized rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Serum Ca (mg/100 ml)</th>
<th>Serum P (mg/100 ml)</th>
<th>BUN (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>9.7 ± 0.2*</td>
<td>8.0 ± 1.3*</td>
<td>21.7 ± 2.2*</td>
</tr>
<tr>
<td>SAT 0.2 g/kg ip (4)</td>
<td>9.0 ± 0.5**</td>
<td>15.4 ± 2.5**</td>
<td>123.6 ± 19.5**</td>
</tr>
<tr>
<td>SAT 0.1 g/kg ip (8)</td>
<td>9.2 ± 0.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SAT 0.05 g/kg ip (8)</td>
<td>9.5 ± 0.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PTX + SAT 0.2 g/kg ip (4)</td>
<td>5.4 ± 0.6†</td>
<td>18.1 ± 3.6</td>
<td>106.2 ± 15.8†</td>
</tr>
<tr>
<td>PTX (6)</td>
<td>5.6 ± 0.3††</td>
<td>13.3 ± 1.8***</td>
<td>20.1 ± 1.3††</td>
</tr>
</tbody>
</table>

SAT was injected intraperitoneally twice a week for 4 weeks.
Number of rats in parenthesis.
Mean ± SEM.
ND: not determined.
* and †† P < 0.001, ** and † P < 0.01 (serum Ca).
* and **; * and *** P < 0.05 (serum P).
* and **; † and †† P < 0.01 (BUN).
Table 3. Effect of Na-sulfacetylthiazole (SAT) and parathyroidectomy on the excretion of urinary inorganic phosphorus (P)

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Mean daily excretion of urinary P (mg) before SAT treatment (A)</th>
<th>Mean daily excretion of urinary P (mg) after SAT treatment (B)</th>
<th>B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact rats (5)</td>
<td>18.8 ± 1.5*</td>
<td>16.6 ± 2.8 §§</td>
<td>0.87 ± 0.21</td>
</tr>
<tr>
<td>Parathyroidectomized rats (5)</td>
<td>8.7 ± 0.8**</td>
<td>7.8 ± 0.9 §§</td>
<td>0.65 ± 0.18</td>
</tr>
</tbody>
</table>

Twenty-four hr urine was collected for 4 days before and after single injection of SAT (0.5 g/kg ip). Mean daily excretion of urinary P before and after SAT treatment and their ratio was calculated in each rat. Number of rats in parenthesis. Mean ± SEM. * and ** P < 0.001, §§ and §§§ P < 0.05.

phosphorus in the parathyroidectomized rats was significantly decreased compared to that of intact rats.

Administration of SAT showed no significant changes in the daily urinary excretion of inorganic phosphorus in both intact and parathyroidectomized rats. There was no significant difference in the ratio of pre and post-SAT urinary inorganic phosphorus between intact rats and parathyroidectomized rats (Table 3).

Effect of SAT on serum calcium of intact and nephrectomized rats

Serum calcium was 9.0 ± 0.1 mg/100 ml in control rats (n = 5); 8.5 ± 0.1 mg/dl in the rats (n = 5) 24 hr after a single intraperitoneal injection of 0.5 g/Kg SAC, 8.4 ± 0.2 mg/100 ml in the rats (n = 5) 24 hr after nephrectomy and 8.6 ± 0.2 mg/100 ml in the rats (n = 5) 24 hr after nephrectomy and 0.5 g/Kg SAT injection. Serum calcium was decreased 24 hr after nephrectomy (p < 0.05). Administration of SAT caused significant decrease in serum calcium in intact rats (p < 0.05) but not in nephrectomized rats. Addition of SAT (80 mM) to rat serum in vitro did not affect serum calcium value.

Discussion

In addition to the pathological changes of soft tissues especially in the cardiovascular system, SAT apparently caused definite atrophic changes in the tail bones and femur of rats as shown by the calcium content, X-ray density and cortical thickness. Histological study and microradiography of those bones of SAC-treated rats revealed bone changes similar to osteoporosis, based on the decrease in the cortical thickness of diaphyses and the increase in the number of bone resorption cavities in the cortex. The prevention of these SAT-induced bone atrophy by prior parathyroidectomy and the increase in size of the parathyroid glands might indicate an important role of the parathyroids in the development of such bone changes.

As a main cause of such bone changes in this experiment, SAT-induced renal failure with increased urea nitrogen and inorganic phosphorus in serum has to be considered. Clinically, osteodystrophy is a well-recognized complication of chronic renal failure varying from rickets or osteomalacia to changes similar to osteoporosis and osteitis fibrosa (Stanbury et al., 1961; Massry et al., 1968). Microradiography of bone in chronic renal failure reveals an abnormally high level of bone resorption and in the majority of patients a reduction in bone formation (Jowey et al., 1969). It is also reported that total or subtotal parathyroidectomy often leads to a reversal of the osteodystrophy in human with chronic renal failure (Stanbury,
In experimental studies, Rutihauser (1936) and Eger (1940; 1949) observed parathyroid hyperplasia and osteitis fibrosa in rats with renal injury by the administration of platinum, copper, lead or uranium and the prevention of such bone lesions by parathyroidectomy. Jowey et al. reported prevention of immobilization osteoporosis in the dogs (Burkhart and Jowey, 1967) and osteoporosis induced by low calcium diet in the cats (Jowey and Reisz, 1968) through prior parathyroidectomy, suggesting an important conditioning role of parathyroids in experimental osteoporosis. The bone changes similar to osteoporosis in the present experiments might have been induced by hyperparathyroidism secondary to renal failure caused by SAT, in view of the preventive effect of prior parathyroidectomy. Absence of a definite increase in the thickness of osteoid tends to exclude osteomalacia, which, furthermore, would not be prevented by parathyroidectomy.

SAT is apparently not a phosphaturic agent by itself, since increase in urinary phosphorus excretion was noted after SAT injection in neither intact nor parathyroidectomized rats. The fact that the administration of SAT caused no significant change in serum calcium of nephrectomized rats might speak against the possibility of a direct effect of SAT on bone or bone metabolism independent of its nephrotoxic effect although no direct in vitro evidence is available. Furthermore, SAT showed no chelating action on calcium ion in vitro. The decreased level of serum calcium 24 hr after SAT treatment is probably due to SAT-induced renal injury. The mortality of the rats treated with SAT for 4 weeks was 50% and prior parathyroidectomy did not increase such mortality.

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References