EFFECT OF ORGANIC GERMANIUM COMPOUND (Ge-132) ON EXPERIMENTAL OSTEOPOROSIS IN RATS

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Abstract—1. The therapeutic effect of organic germanium compound, 2-carboxyethylgermaniumsesquioxide (Ge-132), for experimental osteoporosis was studied using ovariectomized rats maintained on a low calcium containing diet.
2. Serum calcitonin (sCT) level was decreased and serum parathyroid hormone (sPTH) level was increased by ovariectomy and the decrement and increment rates, respectively, were reduced by administration of Ge-132. Thus, the sCT/sPTH ratio was greater in the groups given Ge-132, indicating that the resorption was somehow inhibited by Ge-132.
3. The transverse strength of femur bone was significantly enhanced by Ge-132.
4. A trend was found in the group given Ge-132 for a larger femur conical bone index.
5. The relative femur bone wet weight was greater in the group given Ge-132.
6. These results indicate that Ge-132 prevents decreased bone strength, and affects the femur cortical bone index, and bone mineral mass caused by osteoporosis.

INTRODUCTION

2-Carboxyethylgermaniumsesquioxide (Ge-132) is a low toxic compound (Sugiya et al., 1986a, b) which possesses a wide range of pharmacological effects such as enhancement of osteoblast activity (Orimo and Watanabe, 1985; Watanabe and Orimo, 1986; Nakamura and Orimo, 1987), prevention of mineral decomposition in elderly osteoporosis (Orimo and Akiyama, 1983) and a variety of immunological effects such as a biological response modifier. The present investigation was undertaken to study the pre-clinical effect of Ge-132 on experimental osteoporosis in female rats by performing ovariectomy and feed them a low calcium containing diet.

MATERIALS AND METHODS

Animals
Wistar strain, SPF female rats, 30 weeks old, were purchased from Japan SLC, Inc. (Shizuoka, Japan), and maintained on a refined calcium controlled (Ca 1.0%) semisynthetic diet (No. 1, Table 1) for the duration of the study (-3 to 6 Mo.). Groups 3 to 5 were maintained on a refined calcium controlled (Ca 0.01%) semisynthetic diet (No. 2, Table I) for the first 3 months, and then divided into three groups. Thereafter, Group 3 was maintained on a refined calcium controlled (Ca 0.17%) semisynthetic diet (No. 3, Table 1); Group 4 was maintained on the same diet containing 0.2% Ge-132 (No. 4, Table 1); Group 5 was maintained on the same diet containing 1.0% Ge-132 (No. 5, Table 1), for the duration of the study (0 to 6 Mo.).

At each experimental period of -3, 0, 3, and 6 Mo., a blood sample (5 ml) was obtained from the jugular vein of 10 rats from each group. The serum was isolated and subjected to calcitonin (CT), parathyroid hormone (PTH), and blood biochemical parameter assay. Rats were then sacrificed by decapitation, and right and left femur bones were extracted. Soft tissues surrounding the bone were removed, and wet weight and thickness measured before immersing them into liquid paraffin.

Measurement of blood biochemical parameters, calcitonin, and parathyroid hormone
Serum calcium (sCa: Sarker and Chauhan, 1967), inorganic phosphorus (sIP: Gomori and Ill, 1942), alkaline phosphatase (sALP: Watanabe et al., 1967), and total protein (sTP: Sugawara and Fukushima, 1981) were measured using a Monocard Chemistry System (Amco, Inc., Japan). Serum calcitonin (sCT: Yumita et al., 1985) and parathyroid hormone (sPTH: Fujita et al., 1983) were measured by a method based on radioimmunoassay using the double antibody technique.

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Table 1. Composition of semisynthetic-calcium deficient diet (Modified AIN-Formulation)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>No. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
<td>Ca 1.0%</td>
<td>Ca 0.01%</td>
<td>Ca 0.17%</td>
<td>Ca 0.17%</td>
<td>Ge-132 0.2%</td>
</tr>
<tr>
<td>Corn starch</td>
<td>40.1%</td>
<td>41.5%</td>
<td>41.5%</td>
<td>41.3%</td>
<td>40.5%</td>
</tr>
<tr>
<td>Casein</td>
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<td>25.0</td>
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<tr>
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<td>10.0</td>
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</tr>
<tr>
<td>Cellulose powder</td>
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<td>8.0</td>
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<td>8.0</td>
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</tr>
<tr>
<td>Vegetable oil</td>
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<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
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<tr>
<td>Sucrose</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
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<tr>
<td>Mineral (Modified AIN-76)*</td>
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<td>3.5</td>
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<td>3.5</td>
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<td>Mineral (Modified AIN-76)**</td>
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<td>1.4</td>
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</tr>
<tr>
<td>Ge-132</td>
<td>--</td>
<td>--</td>
<td>0.2</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>100.0</td>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
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</tr>
</tbody>
</table>

*(Mineral composition)

- CaHPO₄
- KH₂PO₄
- NaCl
- K₂H₈O₄
- MgO
- CaCO₃
- Fe₃O₄
- ZnCO₃
- CuCO₃
- MnCO₃
- Fe₂O₃
- Cr₂O₃
- Cr₂(CO₃)₃
- Cu₂O
- ZnCO₃
- CaO
- MgO
- Na₂O
- K₂O
- SiO₂
- P₂O₅
- SO₃
- CO₂
- H₂O
- HNO₃
- H₂SO₄
- HCl
- HC1
- HI
- HBr
- HClO₄
- HNO₃
- H₂O
- H₂SO₄
- HCl
- HC1
- HI
- HBr
- HClO₄
- HNO₃
- H₂O
- H₂SO₄
- HCl
- HC1
- HI
- HBr
- HClO₄
- HNO₃
- H₂O
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- HBr
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- HNO₃
- H₂O
- H₂SO₄
- HCl
- HC1
- HI
- HBr
- HClO₄
- HNO₃
- H₂O
- H₂SO₄
- HCl
- HC1
- HI
- HBr
- HClO₄
- HNO₃
- H₂O
- H₂SO₄
- HCl
- HC1
- HI

***(Vitamin composition)

- Vitamin A
- Vitamin D
- Vitamin E
- Vitamin K
- Vitamin B₁
- Vitamin B₂
- Vitamin B₆
- Vitamin B₁₂
- Folic acid
- Calcium pantothenate
- Nicotinic acid
- Choline bitartrate

Measurement of transverse and compressive strengths

The transverse and compressive strengths of femur bone were determined using the method described by Mizuno (1988). This entailed measuring the strengths using an Instron Universal Type (TOM, 1000X, Shinco Co. Ltd., Japan). Figures 1 and 2 show the sample compartment for the measurement.

Table 2. Protocol for the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental period (months, Mo.)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>OVX</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
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<tr>
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<td>1</td>
<td>OVX</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>OVX</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>OVX</td>
</tr>
</tbody>
</table>

Calcium and phosphorus contents in femur bone

Part of the femur bone was cleaned and then dried at 100°C for 48 hr. A piece of bone (approx. 50 mg) was treated with 2.5 ml of 60% HNO₃ at 110°C for 3 hr. Calcium and phosphorus contents were measured by an atomic absorption spectrophotometer (Model 501, Perkin-Elmer) and Fiske-Subbarow method (Gomori and Ill, 1942), respectively.
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RESULTS

Diet consumption and growth

Although no significant difference was found between diet consumption and growth, lower diet consumption and growth were observed in the group given a low Ca diet and also the addition of Ge-132. The maximum daily consumption of Ge-132 was 375 mg/kg in Group 5.

Blood biochemical parameters

The results of serum biochemical parameters are summarized in Fig. 3. sCa levels decreased from 13.25 ± 0.12 to 12.82 ± 0.17 and 12.79 ± 0.17 mg/dl during the initial 3 months (−3 to 0 Mo.) in Group 2 and Groups 3–5, respectively. They decreased further to reach 12.07 ± 0.16, 12.15 ± 0.12, 12.11 ± 0.28, and 12.25 ± 0.30 mg/dl at 6 Mo. in groups 2–5, respectively. A trend of higher sIP levels, but not a significant difference, was found in Groups maintained on a lower Ca diet and also at higher doses of Ge-132. Also, sALP levels were not significantly different among the groups. sTP levels decreased at 6 Mo., however no significant difference was seen.

Femur cortical bone index

The femur cortical bone index was obtained by the method of Yamamoto and Kishimoto (1983, 1984, 1986). Thus, the radiograph of the transverse section through the distal 1/3 site of the femur bone was obtained using a Sofron Soft X-ray (SRO-M40, Soken Co. Ltd, Japan), and the femur cortical bone index (FCBI) was obtained from the following equation:

\[
FCBI = \frac{d_1 + d_2}{D}
\]

where, \(d_1\) and \(d_2\) are the thickness of the femur cortical bone, and \(D\) is the diameter at the respective site of femur bone.

Statistical analysis

All parameters are reported as mean ± standard errors and analyzed for statistical significance using Student’s t-test and Welch’s t-test for parametric and non-parametric data, respectively. A \(P\) value of < 0.05 was considered significant.

Blood biochemical parameters

Fig. 3. Overview of the sample compartment for the compressive strength test (in mm).
Fig. 4. Effect of Ge-132 on sCT and sPTH levels. From the top, serum calcitonin level (sCT) and serum parathyroid hormone level (sPTH).

Serum calcitonin and parathyroid hormone

The results of sCT and sPTH levels are summarized in Fig. 4. In Group 2, a slight decrease (168.80 ± 25.95 at -3 Mo. to 137.10 ± 11.42 pg/ml at 6 Mo.) was found. The lowered Ca content in the diet (from 1.0 to 0.01%) for the initial 3 months significantly decreased sCT level (Groups 3-5 at 0 Mo.); 163.3 ± 7.8 and 131.5 ± 10.9 pg/ml in Group 2 and Groups 3-5, respectively. The administration of Ge-132 somewhat increased sCT levels and clearly demonstrated in Group 4. On the contrary, sPTH level was increased. In both sCT and sPTH levels, Group 4 gave nearly the same pattern as Group 2, sCT/sPTH ratio was also calculated to demonstrate that Group 2 and Group 4 gave almost the same pattern.

Femur bone weight

The relative femur bone wet weights are summarized in Table 3. The lowered Ca content in the diet for the initial 3 months significantly decreased femur bone wet weight (Groups 3-5 at 0 Mo.): 0.835 ± 0.023 and 0.756 ± 0.008 g in Group 2 and Groups 3-5, respectively. At 3 Mo., Groups 3 and 4 significantly decreased in relative femur bone wet weight compared to Group 2. Group 5 was significantly greater compared with Group 3. At 6 Mo., weights of Groups 3-5 were significantly lower than Group 2, however no significant difference was found in relative femur bone wet weight. The administration of Ge-132 showed a trend of dose-dependent increase of relative femur bone wet weight.

Transverse and compressive strengths

The relative transverse strength is summarized in Table 4. In Group 2, the strength decreased with the elapse of time. The lowered Ca content in the diet for the initial 3 months significantly decreased the relative transverse strength; 56.57 ± 1.56 and 42.83 ± 1.89 in Group 2 and Groups 3-5, respectively. At the experimental period of 3 and 6 Mo., Group 3 showed significantly lower values compared to Group 2. Groups 4 and 5 gave higher relative transverse strength at 3 and 6 Mo. than Group 3. The relative compressive strength is summarized in Table 5 indicating no significant differences among the groups.

Calcium and phosphorus contents in femur bone

Ca content in femur bone is summarized in Table 6. Only a significantly lower value was found in the group maintained on the lowered Ca containing diet for the initial 3 months; 25.8 ± 0.25% and 24.1 ± 0.22%
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Table 5. The effect of Ge-132 on relative compressive strength of rat femur bone

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental period (Mo.)</th>
<th>0</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>225 ± 11</td>
<td>225 ± 15</td>
<td>232 ± 13 (n = 6)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>215 ± 6</td>
<td>224 ± 14</td>
<td>220 ± 18 (n = 5)</td>
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</tr>
<tr>
<td>4</td>
<td>209 ± 4</td>
<td>211 ± 6</td>
<td>227 ± 17 (n = 6)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>219 ± 11</td>
<td>211 ± 6</td>
<td>243 ± 9 (n = 6)</td>
<td></td>
</tr>
</tbody>
</table>

Number in the sample (n) was 8, except where indicated.
Relative compressive strength = compressive strength (g/cm²)/body weight (g).

Table 6. The effect of Ge-132 on Ca content (%) in rat femur bone

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental period (Mo.)</th>
<th>0</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25.8 ± 0.25</td>
<td>25.3 ± 0.16</td>
<td>25.7 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25.4 ± 0.16</td>
<td>24.7 ± 0.25</td>
<td>25.1 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>24.1 ± 0.22***</td>
<td>25.5 ± 0.47</td>
<td>25.9 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>25.0 ± 0.16</td>
<td>25.4 ± 0.16</td>
<td></td>
</tr>
</tbody>
</table>

Number in the sample was 10.
*Compared to Group 2, **P < 0.01.

Table 7. The effect of Ge-132 on P content (%) in rat femur bone

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental period (Mo.)</th>
<th>0</th>
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<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>13.7 ± 0.03</td>
<td>13.8 ± 0.22</td>
<td>13.3 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13.7 ± 0.03</td>
<td>13.7 ± 0.06</td>
<td>13.6 ± 0.22**</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13.8 ± 0.03</td>
<td>13.8 ± 0.03</td>
<td>13.9 ± 0.06***</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>13.9 ± 0.06</td>
<td>13.8 ± 0.06</td>
<td>13.8 ± 0.06***</td>
<td></td>
</tr>
</tbody>
</table>

Number in the sample was 10.
*Compared to Group 2; ***P < 0.001; **P < 0.01; *P < 0.05.

Table 8. The effect of Ge-132 on Ca/P ratio in rat femur bone

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental period (Mo.)</th>
<th>0</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.89 ± 0.03</td>
<td>1.84 ± 0.02</td>
<td>1.92 ± 0.02</td>
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</tr>
<tr>
<td>3</td>
<td>1.86 ± 0.01</td>
<td>1.81 ± 0.02</td>
<td>1.84 ± 0.02**</td>
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</tr>
<tr>
<td>4</td>
<td>1.72 ± 0.01***</td>
<td>1.87 ± 0.03</td>
<td>1.83 ± 0.03**</td>
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<tr>
<td>5</td>
<td>1.79 ± 0.02</td>
<td>1.85 ± 0.02**</td>
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</table>

Number in the sample was 10.
*Compared to Group 2; **P < 0.01; *P < 0.05.

Table 9. The effect of Ge-132 on femur cortical bone index of rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental period (Mo.)</th>
<th>0</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
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<tr>
<td>2</td>
<td>0.26 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.23 ± 0.00</td>
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</tr>
<tr>
<td>3</td>
<td>0.25 ± 0.00</td>
<td>0.20 ± 0.00</td>
<td>0.19 ± 0.01**</td>
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</tr>
<tr>
<td>4</td>
<td>0.18 ± 0.01**</td>
<td>0.22 ± 0.02</td>
<td>0.22 ± 0.01</td>
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</tr>
<tr>
<td>5</td>
<td>0.20 ± 0.00</td>
<td>0.21 ± 0.01</td>
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</table>

Number in the sample was 3.
*Compared to Group 2, *P < 0.05.

in Group 2 and Groups 3–5, respectively, at 0 Mo.
P content in femur bone is summarized in Table 7. A significantly higher value was found in Groups 3–5 compared to Group 2 at the experimental period of 6 Mo. The Ca/P ratio in femur bone was calculated and is shown in Table 8. Compared to Group 2, significant differences were observed in Groups 3–5 at 0 Mo. (P < 0.01) and 6 Mo. (P < 0.05).

Femur cortical bone index

Compared to Group 2, FCBI decreased significantly in the group maintained on the lowered Ca containing diet; Groups 3–5 at 0 Mo. and Group 3 at 6 Mo. (Table 9).

DISCUSSION

For the experimental models of osteoporosis, ovariectomized rats (Izawa et al., 1981), ovariectomized rats on a low Ca diet (Ezawa et al., 1982), ovariectomized rats with hemicordotomy (Okumura et al., 1986), rats on a low Ca diet, a vitamin D deficient diet, rats treated with glucocorticoid (steroid), and rats with a fracture of the tibia, have been investigated extensively.
In general, type I osteoporosis (postmenopausal osteoporosis) is considered to be caused by a decreased blood estrogen level due to incompetence or incontinence of ovary function. Therefore, the method reported by Ezawa et al. (1982) was adopted in the present investigation. Thus, 30 week old female rats were ovariectomized and then maintained on a low Ca diet. By ovariectomy, the relative femur bone wet weight, increased sCT level, decreased sPTH level, constant Ca/P ratio in femur bone were found, indicating that experimental osteoporosis was formed in Groups 3-5. In the case of femur bone transverse strength, the group maintained on a lowered Ca containing diet (Group 3) gave significantly smaller values than Group 2 which was fed a normal Ca containing diet (Ca content, 1.0%). The addition of Ge-132 into the lowered Ca diet resulted in significant increases of transverse strength (Groups 4 and 5) compared to Group 3. In FCBI, Group 3 showed a significantly smaller value than Group 2, but not in Groups 4 and 5. The same trend was found in relative femur bone wet weight. These results suggest that Ge-132 possesses a preferable effect against the decrease of bone strength, cortical bone index, and bone mineral mass caused by osteoporosis.

In all groups, sCT decreased with the elapse of time, with a decrement rate of the following order: Group 3 > Group 5 > Group 4 > Group 2, whereas sPTH increased in the order of Group 5 > Group 3 > Group 4 > Group 2. In considering the calcium regulating factor, sCT/sPTH, these facts indicated that bone resorption might be of the order: Group 3 or Group 5 > Group 4 > Group 2, which agrees with the above results of bone strength, cortical bone index, and bone mineral mass.

The sCT and sPTH levels are regulated by sCa level. Thus, a low sCa level enhances PTH secretion, and a high sCa level enhances CT secretion. These changes of sCT and sPTH, these facts indicated that bone resorption might be of the order: Group 3 or Group 5 > Group 4 > Group 2, which agrees with the above results of bone strength, cortical bone index, and bone mineral mass. In general, it has been reported that the serum biochemical parameters, such as sCa, sIP, and sALP, are normal in osteoporosis in man (Tomita, 1990; Orimo, 1990). In the present investigation, no significant differences in sCa and sIP levels were found, although significant increases in sALP level in Groups 4 and 5 were found at the experimental period of 3 Mo. Since the isozymes of sALP were not investigated, it is not clear whether or not this difference was caused by bone originated ALP.

REFERENCES


