THE RESPONSE OF OSTEOCYTES TO A LIPID CLEARING AGENT IN STEROID-TREATED RABBITS

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We studied the effect of a lipid clearing agent (clinofibrate) on the osteocytes of rabbits treated with corticosteroids. Thirty-one rabbits were divided into four groups: (A) steroid-treated with a normal diet, (B) steroid-treated and on a diet with added clinofibrate, (C) non-steroid-treated, on a diet with clinofibrate; and (D) non-steroid-treated on a normal diet.

All the steroid-treated animals demonstrated hyperlipidaemia and fatty degeneration of the liver. Lipid-containing osteocytes were seen in the femoral heads of these animals. However, those which received clinofibrate (group B) had less severe lipidaemia, and less severe degeneration of the liver. In them, only the osteocytes around the haversian canals exhibited lipid inclusions.

Clinofibrate appears to modify lipid metabolism, diminishing the steroid induced accumulation of lipids within osteocytes. This effect may protect against steroid-mediated osteonecrosis.

One of the most disabling side effects of systemic corticosteroid therapy is osteonecrosis. The pathogenesis of this disorder is uncertain and among the several hypotheses that have been proposed are osteoporosis leading to microfractures (Freiberger and Swanson 1965; Solomon 1973), coagulation abnormalities (Boettcher et al 1970), fat embolisation (Jones and Sakovich 1966; Fisher and Bickel 1971; Fisher et al 1972; Jaffe et al 1972; Cruess, Ross and Crawshaw 1975; Gold et al 1978), swelling of the bone marrow fat cells (Wang et al 1977) and fat necrosis of the osteocytes (Hirohata and Kawai 1983; Kawai, Tamaki and Hirohata 1985). Several clinical and experimental studies have suggested that there may be a connection between osteonecrosis and changes in systemic or local lipid metabolism (Palmer et al 1981; Boskey et al 1983; Hirohata and Kawai 1983; Warman and Boskey 1983; Kawai et al 1985).

Our previous studies revealed abnormal lipid accumulation within the osteocytes in sections from osteonecrotic areas in man, in rabbits with Watanabe hereditable hyperlipidaemia and in rabbits treated with steroids (Kawai et al 1980, 1985; Hirohata and Kawai 1983). These reports all described an enlarged lipid droplet in the osteocyte which compressed the nucleus to one side of the lacuna, and resulted in nuclear pyknosis. It was suggested that lipid accumulation within osteocytes may be an important early pathological change in steroid-induced osteonecrosis.

Suppression of lipid deposition within osteocytes might, therefore, prevent this complication. Clinofibrate (Sumitomo Chemical Co, Osaka), is a recently developed lipid-clearing agent which decreases the serum cholesterol and triglyceride. Its pharmacological effects are thought to be due to inhibition of cholesterol and triglyceride synthesis in the liver (Suzuki 1975, 1976). The present study was designed to investigate the effects of this agent on the steroid-induced accumulation of lipids in osteocytes.

MATERIALS AND METHODS

Experimental model. Thirty-one female Japanese white rabbits, aged three to six months, weighing 2.7 to 3.9 kg, were used. Twenty animals were injected with 5 mg per kg of methylprednisolone (Depo-Medrol, Upjohn, Tokyo) into the gluteal muscles once a week. Eight of them were given a normal diet (group A) and the other 12 rabbits were given a diet in which 200 mg of clinofibrate was added to 140 g of the normal food (group B). Four further animals received the special diet but no corticosteroids (group C) and seven received a normal diet and no corticosteroids (group D). To prevent

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infection, the animals were prescribed 30,000 units of penicillin G (Meiji Seika Co, Tokyo) once a week. The animals were killed by an overdose of Nembutal (Abbott, Chicago, Illinois) at four or six weeks.

Serological tests. Blood samples were collected every two weeks after a 12- to 18-hour fast. Serological tests of liver function included those for glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and δ-glutamyl transpeptidase (δ-GTP). Tests of lipid metabolism were for total cholesterol (TC), and triglyceride (TG).

Table 1. Serological data at the completion of the study in the four groups of animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Transaminase†</th>
<th>Transpeptidase δ-GTP (mIU/ml)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Steroid)</td>
<td>8</td>
<td>191.3 ± 113.7</td>
<td>546.6 ± 448.6</td>
<td>276.7 ± 406.3</td>
<td>301.6 ± 141.5</td>
</tr>
<tr>
<td>B (CF + steroid)</td>
<td>11*</td>
<td>107.6 ± 44.2</td>
<td>161.4 ± 107.6</td>
<td>25.7 ± 17.7</td>
<td>116.6 ± 64.6</td>
</tr>
<tr>
<td>C (CF)</td>
<td>4</td>
<td>20.5 ± 5.4</td>
<td>34.3 ± 10.1</td>
<td>6.3 ± 0.5</td>
<td>16.8 ± 5.9</td>
</tr>
<tr>
<td>D (Normal)</td>
<td>7</td>
<td>31.6 ± 10.8</td>
<td>34.7 ± 15.5</td>
<td>6.0 ± 2.5</td>
<td>82.4 ± 78.7</td>
</tr>
</tbody>
</table>

* one rabbit was withdrawn because of infection
† values expressed as mean ± sd

Histology. Histological sections were from liver tissue and from the subchondral bone in the weight-bearing area of the femoral head. The middle of each femoral head was prepared for transmission electron microscopy and the remaining lateral and medial thirds were fixed in 10% buffered formalin and processed for light microscopy. The femoral heads were then decalcified with 10% formic acid in formalin. The lateral third of the femoral head was stained with haematoxylin and eosin and the medial third was frozen at −20°C with Tissu-Tek (Miles Scientific, Naperville, Illinois) and sliced at about 20µm with a Cryostat (Bright, Huntingdon, England). The frozen sections were washed with phosphate buffer saline (PBS). The surplus fat in the tissue was removed by a brief wash in 50% alcohol. The sections were then immersed in Sudan IV for a few minutes, washed with PBS and embedded with glycerin.

To estimate the lipid content of the osteocytes in the subchondral bone, two sections of each specimen stained with Sudan IV were evaluated. Each section was scored using a modification of Kawai's four grades: 0 = lipid absent, 1 = lipid only around the haversian canals, 2 = lipid in part of the subchondral bone, 3 = lipid throughout the subchondral bone (Owens and Sokal 1961). Fatty degeneration of the liver was estimated from the sections stained with Sudan IV. Each section was scored using four grades: 0 = absent, 1 = mild, 2 = moderate, 3 = severe.

Electron microscopy. Specimens were sectioned into 2 mm cubes with a razor blade and fixed in 10% buffered formalin and 1% glutaraldehyde PBS for 24 hours at 4°C. After rinsing in PBS for 24 hours, the specimens were decalcified in 10% ethylenediaminetetra-acetic acid solution at 4°C for a week. After rinsing in PBS overnight, they were fixed with 2% osmium tetroxide PBS for two hours at 4°C. Specimens were rinsed in PBS and dehydrated in a series of alcohols, placed in 50% propylene oxide and Epon 812 mixture (1:1) overnight, and then embedded in Epon 812. Ultra-thin sections were made from the selected areas with a diamond knife and stained with uranyl acetate and lead citrate. The sections were examined under a Hitachi H-300 electron microscope (Hitachi, Tokyo).

RESULTS

By the end of the study, the group A animals demonstrated an average weight loss of 1000 g and the group B animals had lost an average of 980 g. The group C and D animals gained an average of 400 g. The average daily intake of clofibrate was 150 mg in both group B and group C animals.
Serological results. At the beginning, the experimental and the control animals demonstrated similar serological data but at the end of the study the values for groups A and B had changed. The data are summarised in Table I. The mean values of the liver function tests, GOT, GPT, and δ-GTP in groups A and B are much higher than those in groups C and D which remained within normal limits. Although the group B animals showed increased values for these tests, they were much less elevated than for those in group A.

Group A and B animals demonstrated hyperlipidaemia; group C and D animals had normal serum lipids. Although the group B animals showed hyperlipidaemia, their serum cholesterol and TG levels were much lower than those for group A. The mean value of TC in group B was one-third, and of TG was one-fourth of that for group A.

Histopathology
Liver. No gross or microscopical pathology was identified in the livers of the group C and D animals. The livers in groups A and B were grossly fatty. Histological sections confirmed that the hepatic cells from group A were increased in size and showed nuclear pyknosis with granular cytoplasm containing lipids. The group B livers usually demonstrated slightly enlarged hepatocytes with mildly granular cytoplasm containing lipid droplets. The grades of fatty degeneration of the liver for each group are shown in Figure 1.

Femoral head. Naked eye examination of the femoral heads revealed no abnormality. Light microscopy in group A heads demonstrated thinned bone trabeculae and an increase in the fat cells of the bone marrow. Absence of the nucleus or nuclear pyknosis was seen in the osteocytes of the subchondral bone. In the sections stained with Sudan IV, there was widespread lipid deposition in the osteocyte lacunae (Fig. 2a). Ultrastructural observations confirmed these findings, with lipid

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Fig. 2a
Photomicrographs of sections from femoral heads, stained with Sudan IV (magnification × 50). Figure 2a – A specimen from group A. There is dense staining of the osteocyte lacunae which are all filled with lipids. Figure 2b – A specimen from group B. Only the osteocyte lacunae around the haversian canals are stained. Figure 2c – A specimen from Group D. Normal appearance of osteocyte lacunae. A few positive osteocytes are seen adjacent to the haversian canals.

Fig. 2b

Fig. 2c

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Fig. 3
Electronmicrograph of an osteocyte in a specimen from group A. An intracellular lipid droplet (L) has compressed the nucleus to one side of the lacuna.

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droplets of various sizes seen in the osteocytes of the subchondral bone often compressing the nucleus to one side of the lacuna (Fig. 3).

In the group B femoral heads, empty lacunae and displaced osteocyte nuclei were also observed. However, they were mainly located in the superficial region of the subchondral bone or around the haversian canals. In the sections stained with Sudan IV, the fat-positive material was less widely distributed than in the sections from group A (Fig. 2b) though most haversian canals contained lipids.

The sections from groups C and D demonstrated thick bone trabeculae and many haematopoietic cells in the bone marrow. The osteocytes had round or oval nuclei and did not stain with Sudan IV (Fig. 2c). Some haversian canals contained lipid. The average grades for accumulation of lipid in the osteocytes in each group are shown in Figure 4.

DISCUSSION

Our findings show that the increase in serum lipids which accompanies steroid therapy can be suppressed by clinofibrate, even though the average serum lipid level for group B was still higher than for groups C and D. The liver function tests in group B animals were also better than in group A.

Clinofibrate modified systemic lipid metabolism induced by steroid administration, as evidenced by the moderate fatty degeneration of the liver in group B animals compared to group A and the less widespread lipid accumulation in their osteocytes.

We previously reported the similar effect of clinofibrate on steroid induced lipid accumulation in osteocytes (Kawai, Shimizu and Hirohata 1984). Wang et al (1978) in a similar experiment, focused their attention on the size of the marrow fat cells and not the lipid accumulation within osteocytes. They had previously studied marrow fat-cell size in steroid-treated rabbits and suggested that increase in fat-cell volume caused increased tissue pressure, which in turn diminished perfusion, and caused osteonecrosis (Wang et al 1977). Later, they reported that the effect of a lipid clearing agent was to reduce marrow fat-cell size in steroid-treated animals (Wang et al 1978).

Our study showed that the number of marrow fat cells was highest in group A. Group B animals had fewer fat cells than did those in group A. However, the average marrow fat-cell size did not differ between groups. Recently, Wang et al (1987) have presented similar results to our own, reporting a reduction in the number of osteocytes with large fat vacuoles from 43% in the steroid-treated group to 27% in those treated with a lipid clearing agent.

We conclude that clinofibrate may alter lipid metabolism both locally and systemically and thereby diminish the accumulation of lipids within osteocytes. This effect may protect steroid-treated animals from developing osteonecrosis.

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REFERENCES


