Effect of Berberine on Bone Mineral Density in SAMP6 as a Senile Osteoporosis Model

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The effects of berberine in senescence accelerated mice P6 (SAMP6) were investigated to learn whether the alkaloid affects bone mineral density (BMD). Oral administration of berberine (10 mg/kg/d) to male and female mice for 22 weeks resulted in an increase in BMD in both sexes. A decreased concentration of deoxypyridinoline (Dpd) in urine was only observed in female mice. There was no effect on body or tibia weight or on the concentration of procollagen type I carboxyterminal extension peptide (PICP) in serum.

Key words berberine; senescence accelerated mice P6 (SAMP6); bone mineral density (BMD); deoxypyridinoline (Dpd); procollagen type I carboxyterminal extension peptide (PICP)

Berberine (1), an isoquinoline alkaloid, was isolated from the golden seal, Hydratis canadensis L., a member of the family Ranunculaceae, and also occurs in many other plants including the Berberies species (Berberidaceae) and Aracangelsia flor (Mennnisperrnaceae).1 The pharmacological and antibacterial properties of berberine (1) have been extensively studied and it is widely used as a stomachic and antibiotic. Berberine belongs to the Berberidaceae or the Berberidaceae family and also occurs in many other plants, including the golden seal, Hydratis canadensis L.

The excretion of Dpd, a marker of bone resorption, was measured in the urine samples and analysis was performed according to published procedures of chemiluminescence immunoassay.3 Briefly, the urine sample was centrifuged and stored at −20 °C until assayed. The enzyme immunoassay (ELISA) method for Dpd (pyrilinks-D assay, Metra Biosystems, Inc.) is competitive, and performed in a microplate well strip format, utilizing a monoclonal anti-Dpd antibody coated on the strip to capture Dpd. First, the urinary samples (or standard) with Dpd-alkaline phosphatase were placed in a well, then reacted for two hours in the dark. The well was washed, p-nitrophenylphosphate substrate was added and the captured conjugated Dpd-alkaline phosphatase reaction product was measured at 405 nm. Results are expressed in nanomoles and normalized for the urinary concentration of creatinine, which was determined by an alkaline phosphate method.

**PICP Assay** The serum PICP, a marker for bone formation, was measured 22 weeks after the end of the experiment in serum samples; analysis was performed according to published procedures.9 Briefly, a blood sample was centrifuged at 2000×g for 15 min to obtain serum and stored at −20 °C until assayed. An RIA with polyclonal antibodies against the PICP purified from human skin fibroblasts was used (Orion Diagnostica). The antibody used did not react with fragments of collagen. First, the serum samples or standards with the tracer solution and the diluted antiserum were incubated for 2 h at 37 °C, and the second antibody was added. After 30 min at room temperature, the bound fraction was separated by centrifugation. The supernate containing the unbound tracer was decanted, and the radioactivity of the precipitate containing the bound tracer was counted.

**Statistics** All values were expressed as the mean±S.E.M. of 8—10 animals. Significance of the mean differences in each experiment was analyzed by Student’s t-test, and a p value of <0.05 was considered significant.

![Chemical Structure of Berberine (1)](image-url)
RESULTS AND DISCUSSION

We reported previously that berberine (1) prevented ovariec-tomy-related bone loss in O VX rats after oral administration for 4 weeks at concentrations of 30 and 50 mg/kg/d. It also inhibited both the formation of osteoclasts (OCLs) and the bone-resorbing activity of OCLs in vitro.15

In studies of osteoporosis, O VX rats are usually used as experimental animals10 for the study of postmenopausal osteoporosis. The present studies investigated the effect of berberine (1) on BMD in SAMP6 which can be used as a senile osteoporosis model.7 Senile osteoporosis primarily results from age-related bone loss, implying that net bone resorption exceeds net bone formation.11 Traditionally, age-related bone loss has been attributed to impaired vitamin D activation and decreased calcium absorption.12

When berberine (1) was orally administered at a concentration of 10 mg/kg/d, it significantly improved BMD in both male and female mice compared with the control group (Table 1), but had no effect on body or tibia weight. This indicates that berberine prevents bone loss in senile osteoporosis.

To assess the effect of berberine in bone turnover, we measured two biochemical markers, Dpd and PICP. Bone turnover is characterized both by the formation of new bone and the resorption of old tissue by osteoclasts.13 Dpd is a cross link of bone collagen, which is released and excreted in urine during the process of bone resorption. It has been shown that this bone collagen degradation product, Dpd, is a useful diagnostic marker of osteoporosis and other metabolic bone diseases,14 while PICP is significantly correlated with rates of bone deposition and can be used as a sensitive indicator of bone formation. Bone formation markers indicate osteoblast activity.15 Measurement of the concentrations of Dpd showed that berberine (1) significantly decreased it only in female mice but not in males, and did not influence the concentration of PICP in either sex (Table 2). The level of Dpd in females was significantly higher than in males, indicating that Dpd is more sensitive to change in females than in males.10

As reported, berberine (1) prevented a decrease in BMD in O VX rats and inhibited both the formation of OCLs and its bone-resorbing activity in vitro.5 Therefore, it is suggested that berberine (1) may inhibit bone resorption through OCLs in females, thus inhibiting bone decrease. Berberine (1) decreased the bone loss of males, however, perhaps through a different pathway; this requires further discussion.

In conclusion, our results indicate that berberine (1) inhibits BMD decrease in both male and female SAMP6, and also decreases the concentration of Dpd in female mice.

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REFERENCES AND NOTES

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Table 1. Effect of Berberine on BMD, Body and Tibia Weights in SAMP6

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>34.6±1.4</td>
<td>34.3±1.9</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.052±0.002</td>
<td>0.060±0.002*</td>
</tr>
<tr>
<td>Tibia weight (mg)</td>
<td>39.6±0.7</td>
<td>37.9±1.8</td>
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Administration of berberine was started at the age of seven weeks. Values are the means±S.E.M., n=8—10. Within each row, values with a superscript are significantly different from the control group (p<0.05).

Table 2. Effect of Berberine on Urinary Excretion of Dpd and Serum Concentration of PICP in SAMP6

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
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<tbody>
<tr>
<td>Dpd (mmol/24 h Cr)</td>
<td>22.8±2.8</td>
<td>15.6±2.8</td>
</tr>
<tr>
<td>PICP (ng/ml)</td>
<td>12.0±0.2</td>
<td>12.0±0.3</td>
</tr>
</tbody>
</table>

Values are the means±S.E.M., n=8—10. Within each row, values with a superscript are significantly different from the control group (p<0.05).