AN ACUTE AND FOCAL OSTEOPENIA MODEL USING OVARIECTOMIZED RATS: A RAPID DETECTION OF THE PROTECTIVE EFFECT OF SALMON CALCITONIN

Hiromichi NAKAMUTA, Minoru SASAKI, Masaki ICHIKAWA and Masao KOIDA

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Setsunan University, Nagaotoge-cho 45-1, Osaka 573-01, Japan

A screening method for anti-osteoporotics using ovariectomized rats was designed using a compact method to monitor the bone density. It was found that ovariectomy (OVX) of Wistar female rats (11 weeks old) induced acute and focal osteopenia within 2 weeks, which responded well to intermittent salmon calcitonin (SCT: 5 and 20 U/kg, s.c., every other day) employed as the standard anti-osteoporotic and injected up to 4 weeks with or without a delay of 2 weeks after OVX.

KEYWORDS osteopenia; salmon calcitonin; ovariectomy

INTRODUCTION

The ovariectomized rat is known to develop a menopausal type of osteopenia within weeks\(^1,2\) which may be an ideal model for preliminary screening of anti-osteoporotics in vivo; its combined use with a compact method for quantifying the extent of osteopenia, if available, would provide us a "handy" screening procedure. Nowadays, osteopenia has been monitored using the histomorphometry that Wronski et al.\(^3\) employed to confirm the anti-osteopenic effect of salmon calcitonin (SCT) in rats. The technique provides detailed information on the histopathological state of bone tissue, but only at the cost of a rather lengthy and intensive procedure. In this study, we present a compact method designed to quantify an acute and focal type of osteopenia induced by ovariectomy (OVX) in rats and its successful application for a brief assay of SCT action in vivo.

MATERIALS AND METHODS

Fifty Wistar female rats (10 weeks old) were purchased from SLC (Shizuoka), kept at 23°C on a 12-h light/12-h dark cycle, and fed with normal diet (Oriental MF, Chiba) and drinking water ad libitum throughout the experiment. One week later, 32 were ovariectomized and 12 sham-operated under pentobarbital anesthesia. The rest were untreated controls. The success of OVX was indicated by the disappearance of the estrus cycle on vaginal smear within 2 weeks and later, on autopsy, by the absence of ovaries and atrophy of the uterus. Ovariectomized rats were divided into 5 groups and s.c. treated every other day as follows: (a) with saline (1 ml/kg) from day 1 of OVX to day 13; (b) similarly but to day 27; (c) with SCT (Sandoz: SMC 20-051, 5 U/kg) from day 1 to day 27; (d) similarly but with SCT (20 U/kg); and (e) with SCT (20 U/kg) from day 15 to day 27. Food and water intake and body weight were monitored on the day of treatment. At day 14 or 28, the animal
was killed by exsanguination under deep pentobarbital anesthesia. Tibia and femur were excised, defleshed, defatted in a mixture of chloroform and methanol (1:1) for 24 h, and dried at 100°C for 12 h. From the tibia a proximal 5-mm section and from the femur a distal 5-mm section, in either case comprising the metaphysis-epiphysis, were cut out by a saw. The section was hung from an isometric transducer (Orinect T7-15-240: the transducer may be replaced by a normal gravimeter if the cut phase of the bone is covered by a thin film of paraffin) in such a way that the metaphyseal surface would be kept at the top and weighed first in the air and then in methanol. From weight decrease on complete immersion in methanol, the apparent volume of a section was calculated according to Archimedes's principle. The section was then ashed at 600°C for 12 h, the rest weighed, and the contents of Ca and P estimated by the methods of Gitelman and Fiske and Sabbarow, respectively. Results were expressed as means ± SEM, and the significance of the difference between the two means was assessed by Student's t-test, taking P<0.05 or less as statistically significant.

RESULTS AND DISCUSSION

Within 2 weeks after OVX, the signs of estrogen deficiency developed: increases in body and thymus weights, disappearance of estrus cycle, and so on. Significant losses occurred in the apparent bone density of the sections of both femur and tibia (Fig. 1). The Ca and P contents (g/ml) of femur section, 0.355 ± 0.01 and 0.163 ± 0.004 in the sham group, fell to 0.316 ± 0.006 and 0.146 ± 0.003, respectively, in the OVX group (P<0.01). Most of these changes persisted for another 2 weeks. Intermittent SCT for 4 weeks was

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**Fig. 1.** An Acute and Focal Decrease of Bone Density Induced by OVX in Rats and Effects of SCT. 2W and 4W are the periods after OVX in weeks. : Sham + Vehicle, : OVX + Vehicle, : OVX + SCT 5 U/kg for 4 weeks, : OVX + SCT 20 U/kg for 4 weeks and : OVX + SCT 20 U/kg for the last 2 weeks. Each bar is the mean ± SEM of 5-7 rats. * and ** indicate significant difference between a pair of means connected by a line at P<0.05 and 0.01, respectively.
found to prevent the osteopenic changes in a dose-dependent manner, and prevention appeared to be perfect with the high dose (Fig. 1). The low dose failed to stop the decrease of the Ca content (0.329 ± 0.009) while the high dose kept it unchanged (0.359 ± 0.009). In addition, the high dose was found to normalize the bone density (Fig. 1) and the Ca content (0.357 ± 0.008, P<0.01) which had decreased for the untreated period of 2 weeks after OVX.

This study started when we macroscopically noted a marked loss of cancellous bone in the defatted specimens of tibia from OVX rats and an attempt was made to quantify the bone loss using daily equipment in our laboratory. The method developed then was found to detect a bone loss which occurred within as little as 2 weeks after OVX, as reported by Wronski et al.\textsuperscript{2)} A loss in density of the distal section of the femur (from 1.68 ± 0.02 of the sham group to 1.53 ± 0.02 of the OVX group) was comparable to the value (from 1.589 ± 0.005 to 1.537 ± 0.004) reported by Liu et al.,\textsuperscript{6)} who examined the effect of PTH on ovariectomized rats. The difference between our and their starting values may be due to their use of older rats (95 days old at the start of the experiment) than ours and to the elapse of a longer time between OVX and sacrifice (60 days).

The effect of intermittent SCT was dramatic, and a high dose was found not only to prevent bone loss but also to reverse the one which had existed. A series of experiments to estimate the minimum dosing schedule of SCT required for complete protection is now underway with a study to endorse the findings reported herein histomorphometrically. Some preliminary observations suggest that in most cases the analysis of a given bone specimen by the method described herein can predict the results to be obtained later histomorphometrically.

In conclusion, a combination of ovariectomy with a compact method to quantify osteopenia in rats seems to provide a "handy" screening procedure for the anti-osteoporotic drug in vivo.

REFERENCES


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