Decreased osteoinductive potential of bone matrix from ovariectomized rats

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The effect of estrogen deficiency on matrix-induced bone formation was investigated. Female rats were ovariectomized and given demineralized bone matrix (DBM) intramuscularly 3 weeks before termination. The DBM was taken from previously ovariectomized and from sham-operated on rats. The animals were killed at various times after ovariectomy (6–27 weeks). Implants were processed undemineralized for histologic and biochemical studies.

Normal DBM implanted in ovariectomized or normal rats induced extensive bone formation 6 weeks postovariectomy. The amount of newly formed bone decreased with the age of host rats. Bone matrix taken from ovariectomized rats was incompletely resorbed in both ovariectomized and normal hosts, therefore reducing the extent of osteogenesis and bone-marow formation. Instead, chondrogenesis was intensive, but delayed. The calcium, magnesium, and zinc contents were decreased in implants taken from ovariectomized rats when compared with implants taken from normal animals.

Normal osteoinduction with DBM taken from normal rats and implanted in ovariectomized rats and the absence of osteogenesis with DBM taken from ovariectomized rats indicate that an estrogen-deficient environment is not crucial for altered matrix-induced endochondral bone formation in ovariectomized rats. An altered composition of matrix from ovariectomized rats and a subsequent abnormality in the cell-matrix interaction should be considered responsible.

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The osteoinductive potential of demineralized bone matrix is known to decrease with aging (Syftestad and Urist 1982, Nishimoto et al. 1985) and as a result of conditions that alter skeletal metabolism, such as hypophysectomy (Reddi and Sullivan 1980), vitamin-D deficiency (Vukičević et al. 1986), chronic inflammation (Vukičević et al. 1988), glucocorticoid administration (Rath and Reddi 1979), and magnesium depletion (Schwartz and Reddi 1979). Conversely, androgens (Kapur and Reddi 1989), estrogen and progesterone (Burnett and Reddi 1983), and platelet-derived growth factor (Howes et al. 1988) stimulate bone matrix-induced endochondral bone formation. Whether or not estrogen deficiency alters the osteogenic potential of bone matrix is unknown.

We have studied the bone-inductive properties of matrix from ovariectomized rats.

Material and methods

Donor animals

Thirty-six female Fisher rats (10 weeks old; 140 ± 10 g) were subjected to bilateral ovariectomy or sham surgery. The animals were fed a diet containing 0.5 percent calcium and 0.5 percent phosphorus. They had free access to distilled water. Ten weeks after ovariectomy, the rats were killed by exsanguination under chloral hydrate anesthesia. The effect of ovariectomy was confirmed by autopsy, showing a lack of ovarian tissue and marked atrophy of the uterine horns.

Preparation of demineralized bone matrix

Demineralized bone was prepared as described by Vukičević et al. 1986. Briefly, diaphyses of femora and tibiae from ovariectomized and normal rats were cleansed of the soft tissues, cut into approximately 0.6-cm-long cylinders, freed of marrow, washed in distilled water, absolute ethanol, and ether, and air-dried overnight. Dried bones were then demineralized in 0.6N HCl and stored at –20 °C until use. The inductive potential of bone matrix was tested in young host rats. On Days 14–21 following implantation of demineralized bone matrix, new bone and bone marrow were observed (Reddi and Huggins 1972).

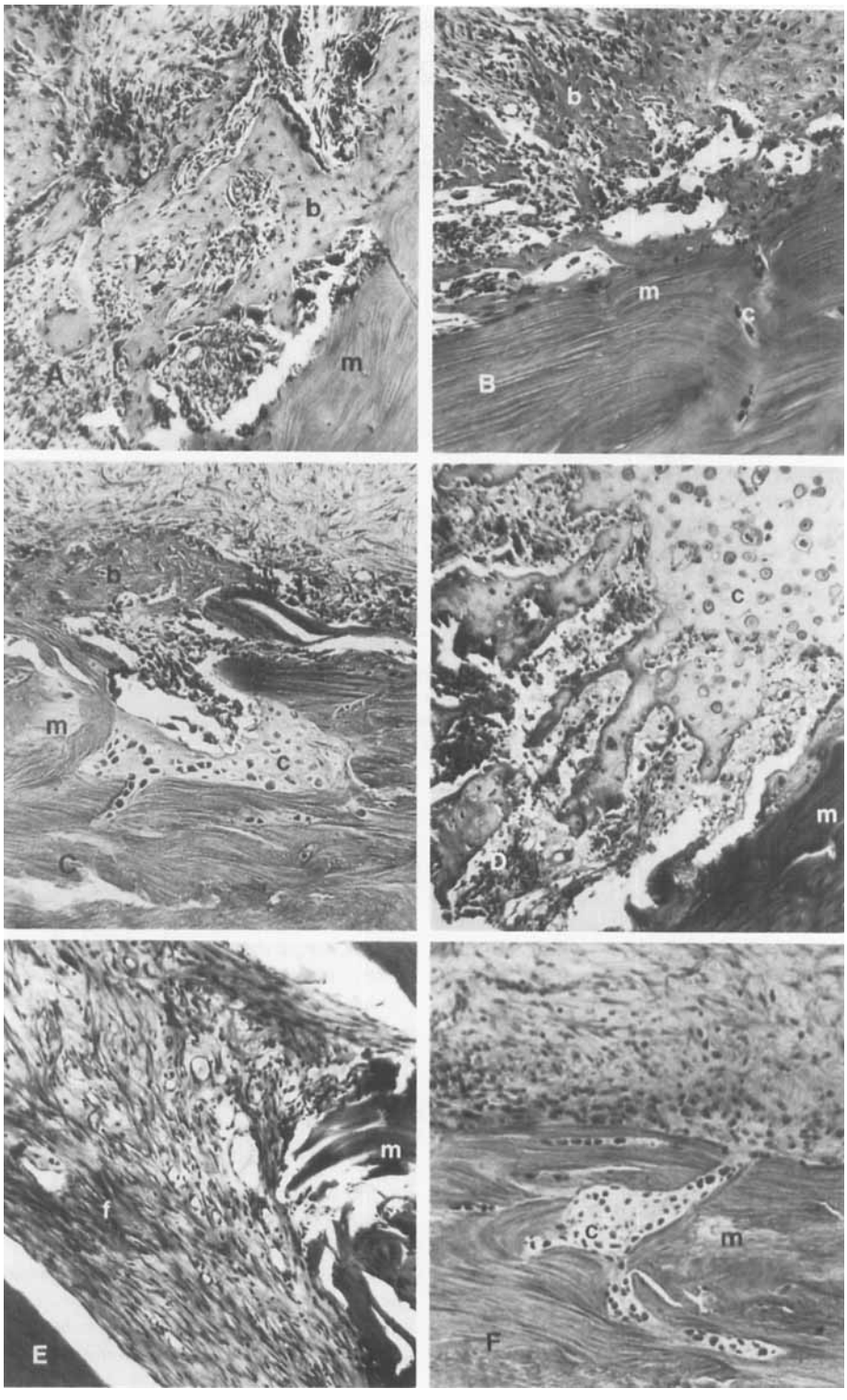

Figure 1 A–F. Normal bone matrix implanted in sham-operated on (S) rat, 6 weeks after the sham operation (A); normal bone matrix implanted in ovariectomized (O) rat, 13 weeks after ovariectomy (B); bone matrix taken from O donors implanted in S rat, 6 weeks after the sham operation (C); 13 weeks after the sham operation (D); 27 weeks after the sham operation (E); bone matrix taken from O donors implanted in O rat, 6 weeks after ovariectomy (F). A–F = 3 weeks after matrix implantation; Goldner's stain, ×100; m old bone matrix, b newly formed bone, c chondrocytic proliferation, f fibroblastic proliferation.

Host animals

Seventy-two female Fisher rats (10 weeks old; 140 ± 10 g) were subjected to bilateral ovariectomy or sham surgery, and were kept under the same laboratory conditions as donor animals. Three weeks before death, two demineralized bone cylinders obtained from previously ovariectomized and two demineralized bone cylinders obtained from previously sham-operated on rats were implanted in separate muscle pouches in the anterior abdominal muscle wall of each animal. The rats were killed at various times after ovariectomy (6–27 weeks).

Six weeks after ovariectomy, the body-weight gain differed between ovariectomized and normal rats (46 ± 7 g vs 21 ± 8 g; $P < 0.05$). From that time onward, the body weight remained stable, and was higher in ovariectomized rats on all the measurement occasions.

Histology

Bone-matrix implants were fixed in 70 percent ethanol and embedded in paraplast without prior demineralization. All the specimens were cut into 3-μm-thick sections (Leitz 1516 microtome, FRG) and stained with modified Goldner's trichrome stain and toluidine blue.

Biochemical analysis

Bone-matrix implants were cleansed of adherent tissues, defatted, lyophilized, and digested with nitric acid in heated quartz flasks as described by Vukičević et al. 1987. Calcium, phosphorus, magnesium and zinc contents were analyzed by (inductively coupled) plasma argon emission spectrometry (Mahanti and Barnes 1983).

A statistical comparison was made using the multifactorial analysis of variance and the Tuckey HSD test (Zar 1974).

Results

Six weeks after the sham operation and 3 weeks after implantation of demineralized bone matrix (DBM), extensive bone formation and bone-marrow accumulation occurred. The amount of newly formed bone decreased with the age of the host animals. Twenty-seven weeks after the sham operation, the implanted bone cylinders were mainly unresorbed, with only a few areas of new bone present. In addition, extensive fibroblastic proliferation in the interior of implanted cylinders was evident. No cartilaginous tissue was found at any time period analyzed. Normal DBM implanted in ovariectomized rats exhibited sequentially similar histologic features to that in sham-operated on rats, with the exception of occasional chondrocytic proliferation within the old matrix at 13 and 20 weeks after ovariectomy (Figure 1).

In sham-operated on rats, 6 weeks after the sham operation, the DBM taken from ovariectomized rats induced less new bone, and bone marrow was absent. Within the old matrix, there were numerous chondrocytes. Thirteen weeks after the sham operation, the interior of the cylinders obtained from ovariectomized rats was filled with cartilaginous tissue, demonstrated by toluidine-blue staining. At 20 weeks after the sham operation, less cartilage was found; and at 27 weeks postoperatively, only fibroblastic proliferation was observed. A similar series of events was apparent when DBM from previously ovariectomized rats was implanted in ovariectomized host animals (Figure 1).

Calcium and phosphorus contents complied with the histologic findings. The mineral content decreased up to 27 weeks after ovariectomy or sham operation in all the analyzed DBM implants. The calcium content was decreased in DBM taken from ovariectomized rats. The magnesium content of all the implants decreased from 13 weeks after ovariectomy or the sham operation onward. The magnesium and zinc contents were decreased in DBM taken from ovariectomized rats (Figure 2; Table 1).

Discussion

In this study, we investigated the potency of bone matrix of estrogen-deficient donor rats in inducing ectopic endochondral bone formation. Our findings

Table 1. Three-way analysis of variance for time of measurements (A), treatment (B), and the type of implants (C)

	Main Effects			Interactions
	A	B	C	A × B × C
Ca	*	NS	*	NS
P	*	NS	NS	NS
Mg	*	NS	*	NS
Zn	*	NS	*	NS

A Six–27 weeks after ovariectomy or sham surgery.
B Control or ovariectomy.
C Bone implants taken from intact or ovariectomized donors.
* $P < 0.05$.
NS Not significant.

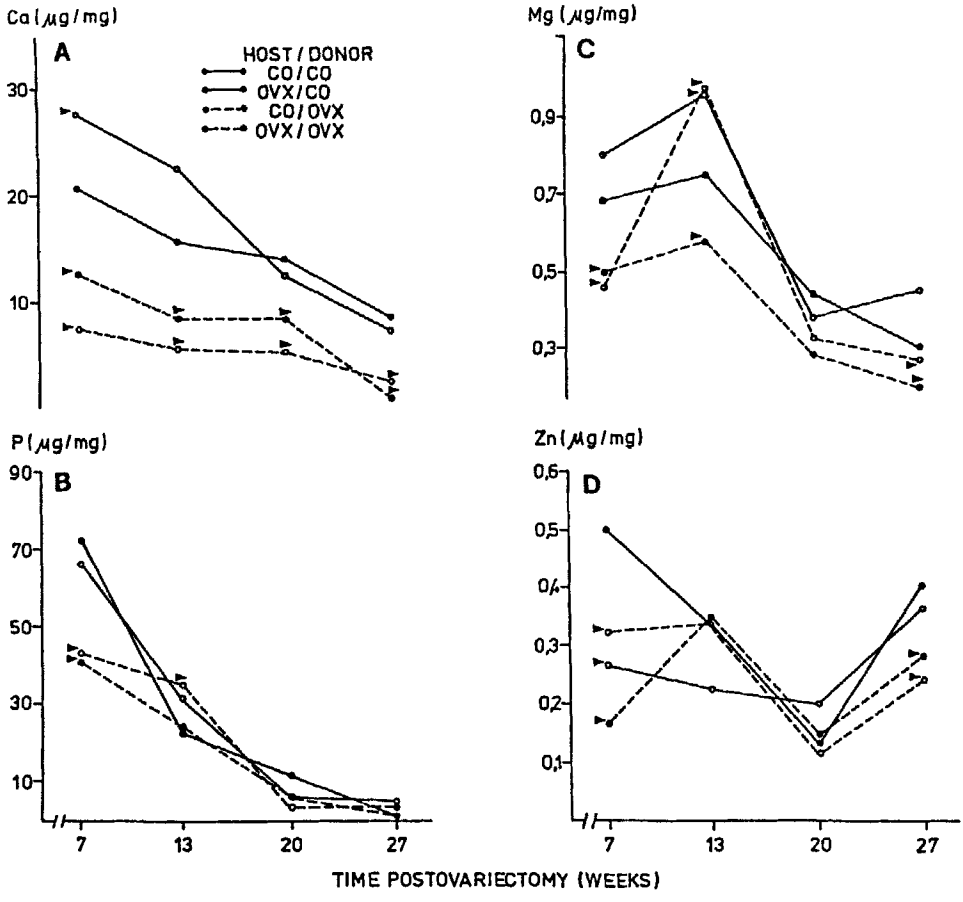

Figure 2. Calcium (A), phosphorus (P), magnesium (C), and zinc (D) contents of bone implants plotted as a function of time postovariectomy. Each data point is the mean of 9 rats; (blank boxes = ovariectomized [O] recipients; black boxes = sham-operated on [S] recipients; full line = implants taken from S donors; broken line = implants taken from O donors). All the SD values were within 9 percent. •$P < 0.05$ vs S/S (see Table 1).

suggest that an estrogen-deficient environment is not crucial for decreased endochondral bone formation in ovariectomized rats. Decreased osteogenesis and delayed chondrogenesis induced by bone from ovariectomized donor rats indicate instead an altered composition of implanted bone matrix and subsequent abnormality in the interaction with host cells.

It has been suggested that bone loss related to estrogen deficiency is partly due to decreased bone formation because of impaired proliferative capacity of bone marrow osteoprogenitor cells. However, the osteogenic potential of marrow stromal cells from ovariectomized rats in vivo has been found to be normal (Tabuchi et al. 1986). When control bone was implanted intramuscularly into ovariectomized hosts, calcium incorporation in the implants was normal 3 weeks after implantation (Tabuchi et al. 1986). These findings support our observation that extensive osteogenesis and bone marrow accumulation were induced when bone from intact animals was implanted in ovariectomized rats at an early stage after ovariectomy. Studies of estrogen-deficiency-related osteopenia have shown increased bone turnover during the initial rapid phase of bone loss, indicating an imbalance between bone formation and resorption in ovariectomized rats (Wronski et al. 1989). Our finding of increased calcium content in normal bone cylinders implanted in ovariectomized hosts supports this transiently increased bone turnover at an early stage after ovariectomy. Bone matrix taken from ovariectomized donors was incompletely resorbed in both sham-operated on and ovariectomized hosts, followed by the reduction of new bone and bone marrow. Instead, chondrogenesis, which normally occurs in the second week (Reddi and Huggins 1972), was abundant and delayed, occurring 3 weeks after implantation.

Although the bone metabolism in rats and humans is different, many qualitative similarities during the course of ovariectomy-induced osteopenia make ovariectomized rats an acceptable model for investigating postmenopausal osteoporosis (Wronski et al. 1989). Results of this study suggest that changes in bone matrix of ovariectomized rats could be responsible for poor fracture healing in the estrogen-deficient environment. Altered production or availability of recently characterized bone-inducing proteins—namely osteogenin (Luyten et al. 1989, Vukičević et al. 1989) and related bone morphogenetic proteins (Wozney et al. 1988)—should be considered a likely cause of impaired osteogenesis in estrogen deficiency.

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