in vivo *21*: 583-586 (2007)

Increased Bone Mineral Density in Aged Rats with Spontaneous Mammary Dysplasia

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Abstract. *Spontaneous mammary tumors were seen in seven of the 12 breeding female rats aged 2 years. All mammary tumors were diagnosed as mammary dysplasia (MD). Bone mineral contents (BMC) and bone mineral density (BMD) of their lumbar vertebrae and femur were determined using dual energy X-ray absorptiometry (DXA). In rats with MD, body weight (BW), BMD of the lumbar vertebrae and BMC of the femur were significantly higher than in the rats without MD. Although corpus luteum (CL) and follicles were seen in the ovaries of all animals, the number of CL in rats with MD was significantly lower than the rats without MD. It was suggested that high BMD, BW and decreased CL promoted mammary tumors.*

It is reported that postmenopausal women have a significant risk of developing a number of debilitating diseases including osteoporosis, breast cancer and cardiovascular disease (1). It is well-known that incidence of osteoporosis is low in breast cancer patients (2). Bone mineral density (BMD) is decreased in postmenopausal women caused by the decreased ovarian estrogen production and serum estrogen, and postmenopausal bone loss can be prevented or arrested by estrogen replacement therapy (ERT) (3, 4). However, it is reported that postmenopausal ERT causes an increase in the risk of breast cancer (5).

In breast cancer patients, osteoporosis is a problem that has been increasingly identified; hormone therapy (tamoxifen) of hormone receptor-positive breast tumors decreases BMD and increases osteoporosis due to the lack of estrogenic effects on bone (6). Obesity, body size and body mass index are associated with an increased risk of breast cancer (7, 8). Low BMD or osteoporosis was recognized in ovariectomized (OVX) rats (9-12). According to previous studies (13-17), rat mammary dysplasia (MD) is induced by 7,12-dimethylbenz-[a]anthracene (DMBA) in neonatally androgenized rats which have no corpus luteum (CL) in the ovaries and maintain a superior level of serum estrogen. We also observed that the induction of MD was strongly suppressed in the androgenized rats that were ovariectomized (15-17). However, the correlation between spontaneous osteoporosis and spontaneous mammary tumors in rats has not been clarified. Therefore, this report investigated the bone mineral density in aged breeding rats with or without spontaneous mammary tumors.

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Key Words: Mammary dysplasia, mammary tumor, bone mineral density (BMD), DXA, rat.

Materials and Methods

Animals. The animals used were 12 inbred Sprague-Dawley (SD) female rats kept in our laboratory for breeding, maintained in a filtered air laminar flow at the Division of Laboratory Animal Science, Research Center for Life Science Resources, Kagoshima University. The animals were given a commercial diet (CE-2, CLEA Inc., Tokyo, Japan) and tap water *ad libitum*. The room temperature was maintained at 25˚C±2˚C and the relative humidity at 55%±10%, with a 12 h light/dark cycle. The use of animals in this research complied with all relevant guidelines set by the Japanese government and Kagoshima University.

Necropsy and tissue preparation. At 2 years after birth, all rats were necropsied by exsanguination from the abdominal aorta under anesthesia with an *i.p.* injection of pentobarbital sodium at dose of 50 mg/kg body weight (BW). The rats were examined for spontaneous incidence of mammary tumors and divided into 2 groups, those with and those without mammary tumors. The mammary tumors and ovaries were removed and fixed in 10% phosphate-buffered formalin. The tissues were then dehydrated and embedded in paraffin. The

Table I. *Diagnosis of mammary dysplasia in rats.*

Group	Animal No.	Number of MD	Number of fibrotic adenosis	Number of acinar adenosis
Rat with MD	1	2	2	0
(n=7)	2	1	1	0
	3	2	1	1
	4	2	0	2
	5	3	1	2
	6	2	0	2
	7	1	0	1
Rat without MD	8	0	–	–
(n=5)	9	0	–	–
	10	0	–	–
	11	0	–	–
	12	0	–	–

MD: mammary dysplasia.

Table II. *Body and organ weights.*

	Rat without MD	Rat with MD
BW (g)	424.8±33.6	467.4±26.1*
Ovaries		
AW (mg)	67.6±9.04	75.9±8.49
AW/BW (mg/g)	0.16±0.035	0.16±0.020
Uterus		
AW (g)	1.12±0.20	0.96±0.16
AW/BW (mg/g)	2.60±0.54	2.10±0.38
Kidneys		
AW (g)	2.22±0.17	2.32±0.18
AW/BW (mg/g)	5.20±0.13	5.00±0.25

(Mean±SD)

MD: mammary dysplasia; BW: body weight; AW: absolute weight; AW/BW: absolute weight/body weight; *$p<0.05$: significantly different from rats without MD.

widest cut surface was sectioned to 5 μm, stained with hematoxylin and eosin and examined histopathologically.

Dual energy X-ray absorptiometry (DXA). From all rats, five lumbar vertebrae (L1-L5) and the right femurs were removed and preserved at –80˚C. Bone mineral content (BMC) and BMD of lumbar vertebrae and femur were determined using dual energy X-ray absorptiometry (DXA, DCS-600A, Aloka, Japan) at Shin Nippon Biochemical Laboratories, Ltd. In the femur, the proximal third, mid-third, distal third, and whole femur were assigned for analysis and expressed as proximal, mid-, distal, and whole femoral BMC and/or BMD, respectively. (9, 10)

Statistics. The mean differences were evaluated using Student's *t*-test.

Table III. *The number of corpus lutea and follicles in ovaries.*

	Rat without MD	Rat with MD
CL	17.0±2.92	13.7 ±1.38*
Follicles	5.2±2.59	6.7±2.21

(Mean±SD)

CL: corpus lutea; MD: mammary dysplasia; *$p<0.05$: significantly different from rats without MD.

Table IV. *BMD and BMC of lumbar vertebrae and femur.*

	Rat without MD	Rat with MD
Lumbar vertebrae 1-5		
BMD	0.146±0.006	0.155±0.005*
BMC	0.470±0.022	0.461±0.019
Whole femur		
BMD	0.142±0.004	0.148±0.006
BMC	0.313±0.018	0.332±0.013
Proximal femur		
BMD	0.138±0.005	0.143±0.005
BMC	0.116±0.007	0.122±0.005
Mid-femur		
BMD	0.141±0.006	0.146±0.006
BMC	0.094±0.005	0.101±0.005*
Distal femur		
BMD	0.147±0.003	0.154±0.010
BMC	0.118±0.011	0.125±0.006

(Mean±SD)

MD: mammary dysplasia; BMD: bone mineral density (g/cm^2); BMC: bone mineral content (g); *$p<0.05$: significantly different from rats without MD.

Results

Mammary tumors were seen in seven of all animals. No other gross findings were observed in any animal. All mammary tumors were diagnosed as MD including fibrotic and acinar adenosis. Multiple MDs were seen in five of seven animals with MD (Table I). BW in rats with MD was significantly higher than the rats without MD ($p<0.05$) (Table II). Although CL and follicles were seen in the ovaries of all animals, the number of CL in rats with MD were significantly lower than the rats without MD ($p<0.05$). The number of follicles was not significantly different (Table III). BMD of the 1st-5th lumbar vertebrae ($p<0.05$) and BMC of the mid-femur ($p<0.05$) in rats with MD were significantly higher than the rats without MD (Table IV).

Discussion

All of the animals were thought to be postmenopausal because menopause begins at 15-18 months of age in rats

(19, 20). The present study revealed that CL and follicles were seen in the ovaries of all animals. However, the influence of the CL in rats with MD may have decreased because of the lower number of CL.

Ovariectomy led to a depletion of serum estrogen and a decrease of BMD in rats (9-12). In the present study, rats without MD were considered to be in a condition of decreasing serum estrogen concentrations because of their low BMD.

In postmenopausal or OVX women, obesity has been associated with an increased risk of breast cancer because androgens produced in adrenal glands were converted to estrogen by adipose tissue (21-23). In OVX rats, adipose tissue contributed to the extragonadal aromatization to promote serum estrogen levels (24). In the present study, it is possible that rats with MD had increasing serum estrogen concentrations because of aromatizing activities from their high body weight.

In our previous study (13-18), the superior level of serum estrogen has accelerated to DMBA-induced MD in rats. In the present study, it is also considered that a superior level of serum estrogen concentration has accelerated to spontaneous MD in aged rats.

In conclusion, it was suggested that high BMD and BW and decreased CL promoted spontaneous mammary dysplasia in aged rats. It is considered necessary to further investigate serum levels of sex hormones.

Acknowledgements

This work was supported in part by Kodama Memorial Fund Medical Research. We are grateful to Mr. T. Kodama for his valuable technical assistance.

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Received January 25, 2007
Revised March 23, 2007
Accepted March 28, 2007