The effects of organic nitrates on lumbar spine bone mineral density and narrow blood perfusion in ovariectomized female rats.

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Introduction: The common pharmacologic agents to prevent osteoporosis include estrogen replacement therapy, bisphosphonates, and selective estrogen receptor modulators (raloxifene). Each medication has adverse effects, often resulting in discontinuation. Several lines of evidence suggest that nitrates, drugs typically prescribed for the treatment of angina, may be effective in preventing postmenopausal osteoporosis. Nitrates have several advantages over the medications currently used to prevent and treat osteoporosis (1). There have been no reports of an increased risk of cardiovascular disease or breast cancer with long-term use of nitrates (1). Nitrates are generally more available worldwide, more convenient to take, and less expensive. However, the mechanism of how nitrates influence bone mechanism remains unclear. Ovariectomy (OVX) of female rats represents a classical osteoporosis animal model. It has been reported that in addition to the decrease of lumbar spine bone mineral density (BMD), there is also a decrease of bone marrow perfusion in the lumbar vertebral bodies (2). Its mechanism is not fully understood yet, but a reduction of erythropoietic marrow and an increase of marrow fat in OVX-ed rats, as well as endothelial dysfunction may be at least partially contributed to this bone marrow perfusion decrease (2). This study investigated the relation among the effect of nitrates on bone marrow perfusion and bone mineral density (BMD).

Materials and Methods: 14 matured SD female rats (6 months old) were used in this study, including OVX group (n=6) and OVX+ISM group (n=8). The organic nitrate used in this study was isosorbide-5-mononitrate (Is-5-Mn, ISM). ISM was dosed orally 150 mg/kg per day, b.i.d till the end of the study (3). CT bone densitometry and perfusion MRI were performed baseline and 8 week post ovariectomy. BMD was measured by using a clinical multidetector computed tomographic (CT) scanner (4). MR imaging was performed by using a 3-T clinical MR imaging system. Rats were anesthetized, and a 24-gauge heparinized catheter was inserted into the tail vein. A dedicated quadrature volume RF coil of 7 cm internal diameter was used as signal transmitter and receiver. Rats were placed in the coil supine, and a central sagittal plane was prescribed, and the following dynamic MR scan series was obtained: gradient echo sequence, TR=5.4 msec, TE=2.3 msec, flip angle=12, slice thickness=2 mm, acquisition resolution=0.63x0.63 mm, temporal resolution=0.6 sec/acquisition, average=1. MRI contrast agent was Gd-DOTA (Guerbet). A dose of 0.15 ml/kg (0.075 ml for a 250 gram rat) was hand-injected after initial baseline 60 image acquisitions as quick bolus and followed by a flush of 0.5 ml normal saline. The DCE MRI scan duration was 8 min. Maximum Enhancement (EM) was used in this study representing blood perfusion, it was defined as the maximum percentage increase in signal intensity from baseline, and calculated from the signal enhancement curve of the dynamic MRI scan (2,5). To obtain EM, regions of interest were drawn over the cancellous part of the lumbar vertebrae from L2 to L5, excluding the vertebral cortex. At the end of in vivo study, for histologic examination lumbar spines were excised and fixed in 10% buffered formalin for 4 weeks. Decalcified samples were embedded in paraffin and cut into 6 mm-thick axial slices. Slices were stained with hematoxylin-eosin. For each rat, four vertebral slices from each of the four vertebrae (L2 through L5) were randomly selected for evaluation. Percentage area of marrow fat, and fat cell number per field (magnification×200) was measured in each histologic slice by using image processing software (Image-Pro Plus, version 5.1; Media Cybernetics). The histology results from this study were compared with results from 10 age matched SD female control rats.

Results: In the OVX group, lumbar spine vertebral BMD decreased by 22.5±5.7% (from 0.93±0.15 to 0.7±0.09 g/mm³, p<0.001 baseline vs week 8), while in the OVX+ISM group lumbar spine vertebral BMD decreased by 15.8±3.5% (from 1.06±0.21 to 0.89±0.17 g/mm³, p<0.001 baseline vs week 8) (Fig 1). The difference between the two groups was significant (p<0.05). In the OVX group, ME decreased by 7.7% (from 62.1±6.4% to 57.9±5.2%, p<0.05 baseline vs week 8), while in the OVX+ISM group lumbar spine vertebral BMD decreased by 12.5% (from 67.3±6.6% to 54.8±5.1%, p<0.01 baseline vs week 8) (Fig 1). The difference between the two groups was significant (p<0.05). Histology results demonstrated OVX rats had the most amount of fatty marrow (therefore least amount of red marrow), and control rats had the least amount of fatty marrow (therefore most amount of red marrow); while the marrow of OVX+ISM rat lied between OVX rats and control rats (Fig 3). The difference between control rats and OVX+ISM rats, and OVX+ISM rats and OVX rats were both significant (p<0.01).

Discussion and Conclusion: It has been known rats between the age of 6 months and 8 months old maintain tend to maintain their lumbar spine BMD and marrow perfusion consistent (5). This study shows ISM orally administered 50 mg/kg per day b.i.d partially prevented the lumbar spine bone loss due to OVX, and maintained the blood perfusion in lumbar vertebral marrow. Vertebral marrow composition reflected these changes. The animal number used in this study is small, further studies, including adjusting ISM dosage, are required to validate these findings.


Acknowledgement: Supported by a grant a direct grant for research of The Chinese University of Hong Kong (2041501) and Hong Kong GRC grant SEG_CUHK02