# Effects of Heavy Ion Particle Irradiation on Bone Metabolism of Rats at Different Ages

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## INTRODUCTION

In early studies it was observed that the effects of radiation such as X-, Gamma ray on bone growth were related on the age at exposure and to the radiation dose. Bone growth stunting by irradiation was more effective in the young and was more pronounced after higher radiation dose. Recently, heavy Ion Medical Accelerator in Chiba (HIMAC) in our institute was developed in order to treat a cancer therapy and while the fundamental researches on radiation biology including our interesting space radiation effects have been carried out. However, the effects of heavy ion particle irradiation on the body are not known because such researches have just started.

The purpose of this present study was to clarify the dose-response and age-response on bone tissue and bone mineral density in rats with the heavy ion particle irradiation.

## MATERIAL AND METHODS

### *Animals*

A total of 120 female Wistar rats were used. The animals were 3, 6, 9, 12, 18 and 32 months old at start of irradiation. 5 rats were housed per standard rat cage and maintained in an air-conditioned room, temperature 22±2, relative humidity 55±5%, and 12h light-dark cycle. The animals were fed with a pellet diet and water *ad libitum*.

### *Irradiation*

For heavy ion particle irradiation, the radiation factors were : Carbon ion beam 290MeV, LET; 40 keV/μm.

The animals were exposed to heavy ion particle in Lucite radiation exposure chambers. The rats in each age group (n=20) were subdivided into four groups of consisting 5 animals and three groups of them were received a single whole body irradiation with doses of 1.25, 2.5, and 5 Gy by the HIMAC. And they were sacrificed 3 months after the irradiation.

### *Bone histomorphometry*

For vital staining, the rats were received twice injections of 25mg tetracycline per kg body weight at intervals of 7 days. After killing the rats with either, the soft tissue was gently removed and the tibia was fixed in 70% alcohol solution and immersed in Villanueva's bone stain solution, dehydrated in alcohol and embedded in methylmethacrylate. The block was cut on a Leitz sawing microtome (10μm thick sections), and mounted on glass slide. The bone histomorphometric analysis was performed by a semi-auto image analyzer with the software "Osteoplan" (Carl zeiss).

### *Bone mineral density*

The bone mineral density of tibia at the proximal metaphysis and diaphysis at the site of 3 and 12mm from the growth plate, respectively was measured with a peripheral quantitative computed tomography (Stratec and Norland). And bone stress/strain index of tibia cortical region was led by a calculation.

### *Serum biochemical analysis*

Serum rat- PTH, CT, Ca were measured.

## RESULTS

The bone mineral density in the trabecular bone and bone stress/strain index of cortical bone in the control groups increased from 3 to 6 months and reached the peak, and then tended a very moderate decrease with age (Figs. 1 and 2). The values in the irradiated groups were varied at each age. The bone mineral density at 3 and 6 months, and the bone stress/strain index at 3, 6 and 9 months. However, the each value in the irradiated groups showed rather higher than those in the control groups, although there were significant differences. At 9 months, the both values decreased with the doses (Fig. 3).

Then, the bone histomorphometric analysis was performed using undecalcified specimens of the tibia at 9 months of age. The bone volume/bone tissue decreased with age, and fitted with the curve of bone mineral density (Fig. 4).

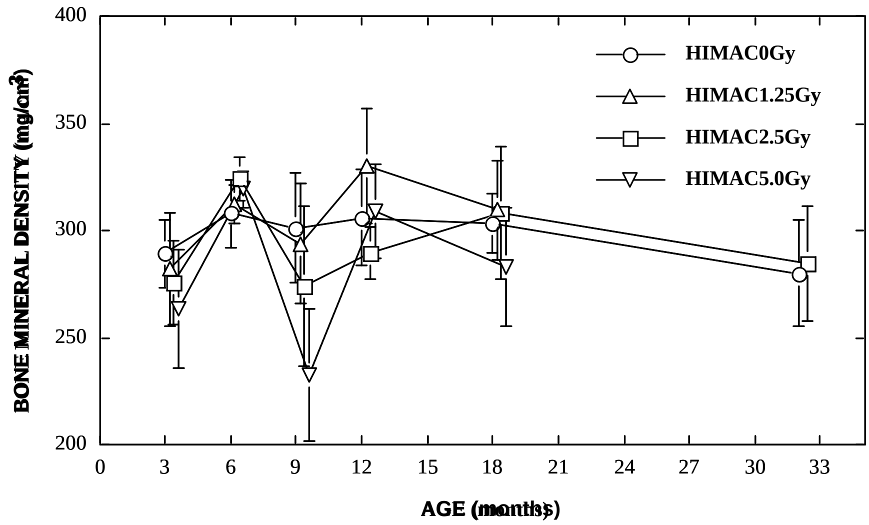


Figure 1. Bone mineral density in the trabecular bone of tibia at 3 months after heavy ion particle irradiation as a function of exposure age for different exposure dose.

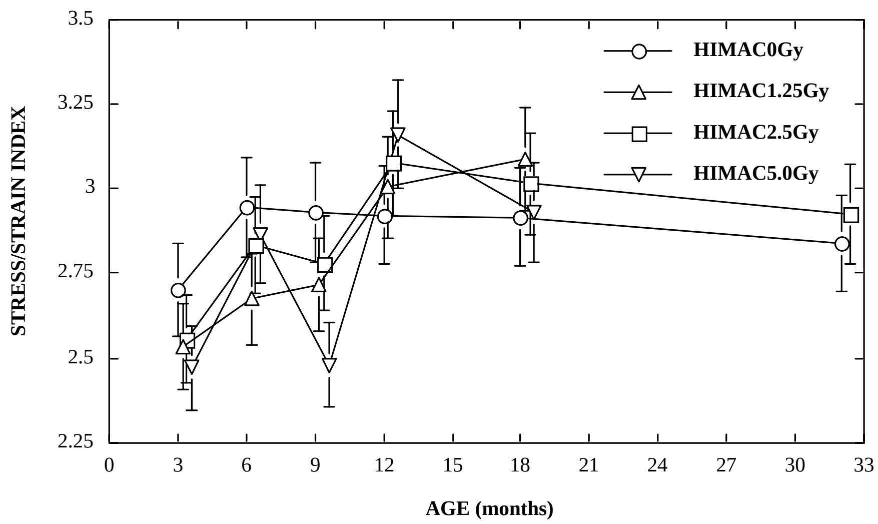


Figure 2. Bone stress/strain index in the cortical bone of tibia at 3 months after heavy ion particle irradiation as a function of exposure age for different exposure dose.

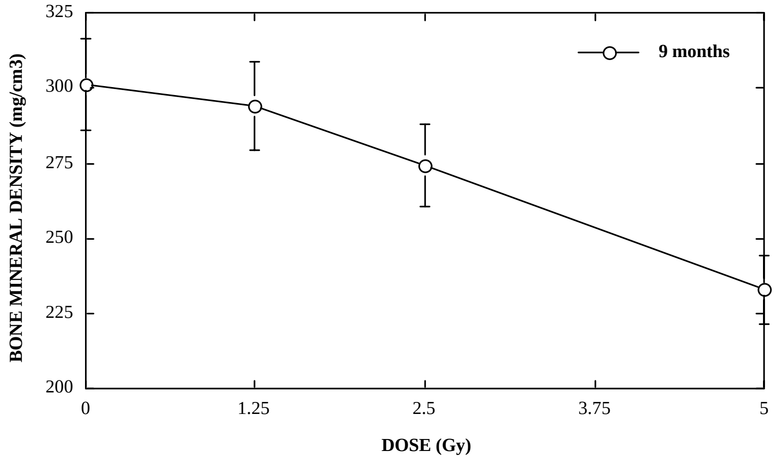


Figure 3. The relationship doses and bone mineral density in the tibial proximal metaphysis 3 months after heavy ion particle irradiation at the age of 9 months in rats (shown in Fig. 1).

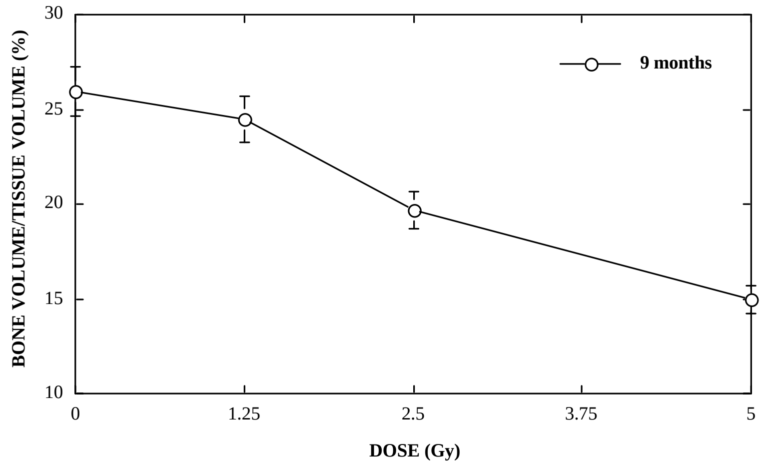


Figure 4. The relationship doses and bone volume (trabecular bone volume/tissue volume) in the tibial proximal metaphysis, contrast to the data shown in Fig.3.

# DISCUSSION

We demonstrated that the bone mineral density and bone volume increased to 9-12 months old and thereafter moderately (very slowly) decreased with age, as shown those in the control groups in Figures 1 and 2. The small or almost no differences in the these values at 3 and 6 months may be due to cover with the bone growth or recover during the period of 3 months from the irradiation till the bone observation. The higher value of bone stress/strain index than those in the irradiated groups of more than 12 months old may be probably due to atrophy of cortical bone.

The data obtained from 9 months old indicated that there was dose-response on bone metabolism, resulting in the decrease in bone mineral density and fragility of bone after irradiation. The reason why the dose-response curve was obtained might be that bone formation and bone resorption was balanced at the age. At the younger age than this age, bone fromation was supeior to bone resorption, while at the older age the bone formation was lessor than bone resorption. That is, at the 9 months old, radiation effects appeared more strength to osteoblast, a cell of forming bone and mineralization, compared to the ostoclast, because the osteoblast is radiosensitive more than osteoclast.

Any way, bone metabolism was affected by the heavy ion particle irradiation in the radiation therapy and space. However, the attributions of influences by other organs related to the bone, e. g., calcium regulating of parathyroid, calcium absorption in the small intestine, calcium excretion of kidney and radiation stress of adrenal gland. Now we are advancing to obtain the detailed data to assess the effects of heavy ion particle irradiation.

## REFERENCES

1. R.D.Philips, D.J.Kimeldorf, *Age and dose dependence of bone growth retardation induced by x-irradiation.* Radiat Res. 27(3), 384-96(1966)