MAGNETIC RESONANCE SPECTOROSCOPIC (MRS) ANALYSIS OF THE OSTEONECROTIC INTERCALATED BONE

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INTRODUCTION:

In diagnosis of the osteonecrosis, such as Kienbock disease, magnetic resonance imaging (MRI) is widely used to detect low signal intensity area in the osteonecrotic intercalated bone. However, what the low signal intensity area on MRI objectively indicates has not been well described. In the present study, we examined the necrotic bone *in vitro* using the line scan magnetic resonance spectoroscopy (MRS) in the series of animal necrotic bone model. Fat/water content of the necrotic lunate was calculated with Carr-Purcell-Meiboom-Gill (CPMG) line scan MRS.

METHODS:

***Animal model*:** Twenty-four Japanese white rabbits were evaluated in this study. The 4th tarsal bone of the rabbit was resected and dipped into the liquid nitrogen to make complete cellular necrosis (1). We replaced the tarsal bone into the intraarticular space of the knee. After 3-days, and 1,2,3,4,8,12,16,20-weeks after the immersion to the liquid nitrogen, rabbits were sacrificed to remove the tarsal bones for MRI, MRS and histologic study.

Analysis of magnetic resonance spectoroscopy: All MR scan and MRS were obtained by 1.5-T Sigma imager (GE Medical Systems, Milwaukee). A 5-cm transmit/receive extremity coil using linearly polarized radio-frequency (RF) pulse was used. The basic spectroscopic imaging sequence consisted of Carr-Purcell-Meiboom-Gill (CPMG) echo trains (2, 3), in which the section-selective excitation pulse was set on an orthogonal plane that was refocused by the 180° RF pulse, as in the classic inner-volume imaging methods (4). Frontal view was taken through the targeted spectroscopic column (dark vertical band), which was outlined with the saturation pulse sequence (**Fig 1b**). Then the signals of the water and fat content were extracted, where the lipid was set in right and the water left. Arrow indicated regions where the spectra were extracted. Pixel numbers along the x axis have been converted to parts per million, with water appearing by approximately 4.8ppm, fat appearing by approximately 8.3ppm (**Fig 1d**). Top spectrum was usually from entirely bone marrow. The logarithm of the peak area versus TE was fit with a linear least-squares method to estimate individual water and fat T2 values and to extrapolate peak areas at zero TE for both water (Pw) and fat (Pf). The relative percentage of water within the voxel was then estimated by using the follwing formula,
where percent water =[Pw/(Pw+Pf)]*100, with percent lipid=100-percent water.

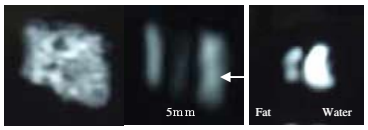

Fig 1a **Fig 1b** **Fig 1c**

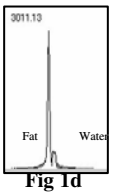

Fig 1d

Histologic analysis: The osteonecrotic bone was embedded in paraphine, decarcified, then stained with hematoxylin-eosin (HE) and Villaneuva Goldner staining. Light microscopic analysis was carried out.

RESULTS:

Postoperative fat/water ratio with the line scan MRS decreased to 19.0% at 3 days, 16.4% at 1 week, 15.9% at 3 weeks, 8.9% at 3 weeks, 6.2% at 8 weeks, 21.8% at 12 week, 14.4% at 16 weeks, and 28.7% at 20 week, respectively, when compared with the control of 82.4% (**Fig 2**). The histologic findings in hematoxylin and eosin (HE) and Villaneuva Goldner staining demonstrated the complete defect of osteocyte nucleus and decrement of osteoid-osteogenesis at 3-8 weeks after the liquid nitrogen immersion (**Fig 3, 4**).

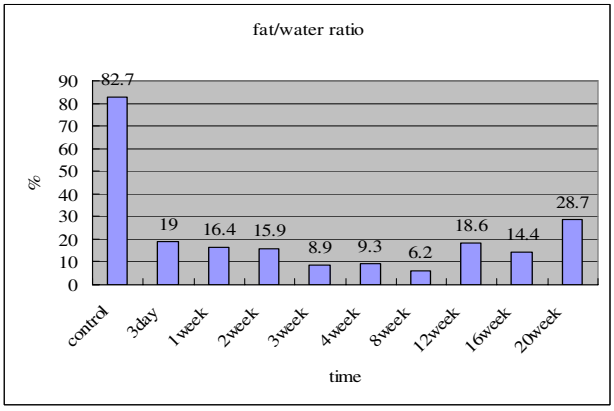

Fig 2: Fat/Water ratio of the necrotic bone

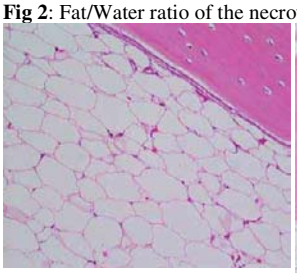

Fig 3a: HE stain of control

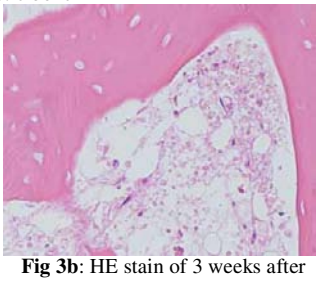

Fig 3b: HE stain of 3 weeks after immersion

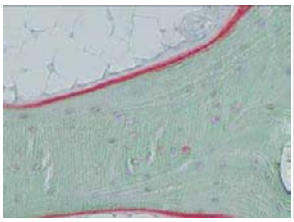

Fig 4a: Villaneuva Goldner stain of control

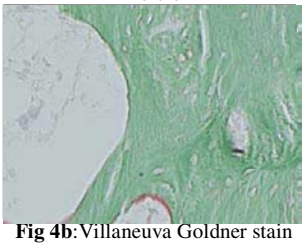

Fig 4b:Villaneuva Goldner stain of 3 weeks after immersion

DISCUSSION:

From the data of the present MRS study, the fat/water ratio of the intercalated bone decreased soon after the immersion into the liquid nitrogen, which was strongly induced the tarsal bone to the osteonecrosis confirmed by the histologic sections of HE and Villanueva Goldner stainings. Decrease of fat/water ratio on MRS was noted relatively faster than histologic changes of bone necrosis. After long term follow up, the fat/water ratio increased to 28.7%. What the low signal intensity on MRI of the necrotic disease may be decrease of fat content in the bone marrow. MRI can delineate loss of fat in the bone marrow in the early stage of Kienbock disease. We also confirmed the line scan MRS, which can evaluate accurate value of fat content in the bone, was accurate diagnostic method and recovery evaluation for necrotic disease *in vitro* or *in vivo*.

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