Magnetic Resonance Imaging of Creeping Substitution in Grafted Bone

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ABSTRACT. Using grafted bone from a rabbit, we investigated experimentally whether creeping substitution of grafted bone is detectable by Magnetic Resonance Imaging. The bone marrow/muscle ratio in T1-weighted images showed a correlation with the ratio of fat cells. The T1-weighted images of the free grafted bone initially showed a high signal intensity due to the high ratio of fat cells. This signal intensity decreased due to new bone formation. Therefore, T1-weighted images can demonstrate histological changes in grafted bone.

Key words: MRI — bone graft — creeping substitution

Magnetic Resonance Imaging (MRI) is useful in the early diagnosis of avascular necrosis of the femoral head,[1] but the correlation of MRI findings with the histopathological stage has not been clarified. In the present study, using free grafted bone and vascularized grafted bone, we investigated experimentally whether creeping substitution of grafted bone is detectable by MRI.

MATERIALS AND METHODS

Fifteen adult rabbits were used in two different experiments. Intravenal pentobarbitone anesthesia was employed. The rabbits were studied prospectively by mobilization of 3 cm of the diaphysis of the bilateral tibia through an anterior approach. Two osteotomies were performed through the diaphysis of each tibia with an oscillating saw. The posterior muscle pedicle of the left tibia, which contains nutrient arteries, was divided and a 3 cm segment of the tibia with its periosteum was removed. Then the devascularized segment was replaced in its original bed, and the soft tissues were closed.

The same procedure was carried out on the right tibia, but the posterior muscle pedicle containing the nutrient arteries was not divided. The limb was fixed using an above-knee cast with the knee in 90° of flexion and the ankle in the neutral position. The healing status of the autograft after each procedure was investigated at one, two, four, six and eight weeks.

Magnetic resonance images were recorded by a Yokogawa Medical Resona Plus superconductive magnet using a spin-echo technique at 0.5 tesla. Coronal images centered on the grafted bone were used for this study (Fig. 1). We obtained T1 calculated images from spin echo at a repetition time of 30 msec

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and an echo time of 25 msec. The pixel size was 0.78 mm. After setting the region of interest in the display, the T1 values of a 0.2 cm^2 area in the center of the grafted bone marrow and the surrounding intact muscle were measured. Wilcoxon's method as used in the Rank Sam Test was employer as a nonparametric statistical method, with the STAX computer software program.

At sacrifice, both legs in each rabbit were retrieved by disarticulation at the knee and ankle and fixed in 10% buffered formalin. The fracture union at both ends of the grafts was assessed clinically as well as radiologically and histologically. The soft tissues were removed from the tibia to expose bare bone and radiographs were taken.

Decalcified specimens were used for histological examination of the grafted bone. The specimens were cut into longitudinal sections 6 μm thick and then were stained with hematoxylin and eosin. The ratio of fat cells in the tissue specimens was determined by an IBAS-2000 graphic analyzer (CARL ZEISS Co.).

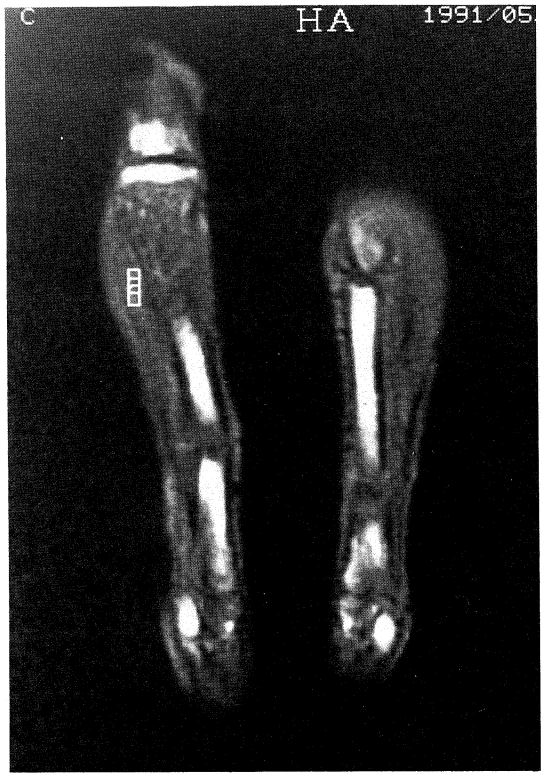

Fig. 1. The T1-weighted MRI

The right tibia is vascularized bone graft.
The left tibia is free bone graft.

RESULTS

The bone marrow/muscle ratio of T1 values for the free bone graft was higher than that for the vascularized bone graft during the early period, but at four weeks they became the same. Subsequently, after six weeks, the ratio was higher in the vascularized bone graft. A significant difference between the two grafts was noted only at two weeks after grafting ($p<0.05$) (Fig. 2). The free bone grafts took about six weeks to achieve osseous union, while the vascularized bone grafts took only four weeks to achieve complete union.

Histological examinations revealed osteonecrosis up to two weeks after grafting in the free bone graft, with growth of granulation tissue, proliferation of blood vessels and osteoblasts. New bone formation was observed after six weeks. In the vascularized bone graft group, reactive hyperemia and infiltration of inflammatory cells were present up to two weeks after grafting, but there was no evidence of osteonecrosis. The appearance of the bone marrow in the vascularized bone graft was normal after four weeks.

The ratio of fat cells in the bone marrow of the tissue specimens of the free bone graft group did not markedly change up to four weeks, but tended to decrease after six weeks. In the vascularized bone graft, the ratio tended to increase up to four weeks, but did not change after six weeks.

The correlation coefficient of the bone marrow/muscle ratio for the T1-weighted images and the ratio of fat cells in the tissue specimens was 0.726 ($y=0.015x+1.369$) at the 1% significance level in the free bone graft (Fig. 3), and 0.879 ($y=0.041x+0.094$) at the 0.1% significance level in the vascularized bone graft group (Fig. 4).

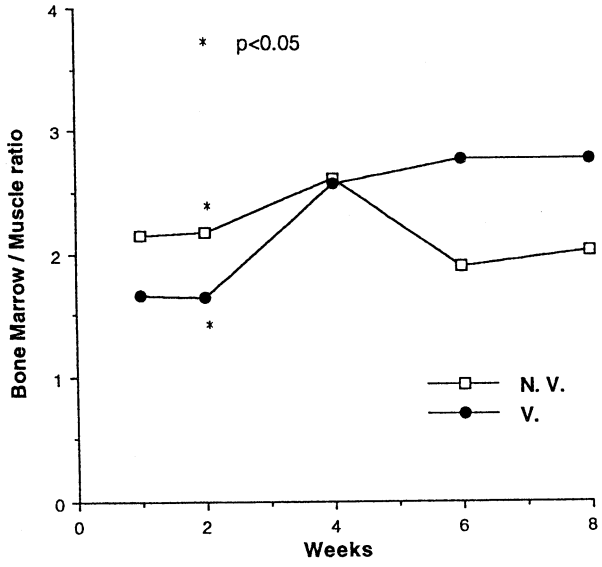

Fig. 2. Signal intensity of the T1-weighted images
N.V. is the free bone graft.
V. is the vascularized bone graft.

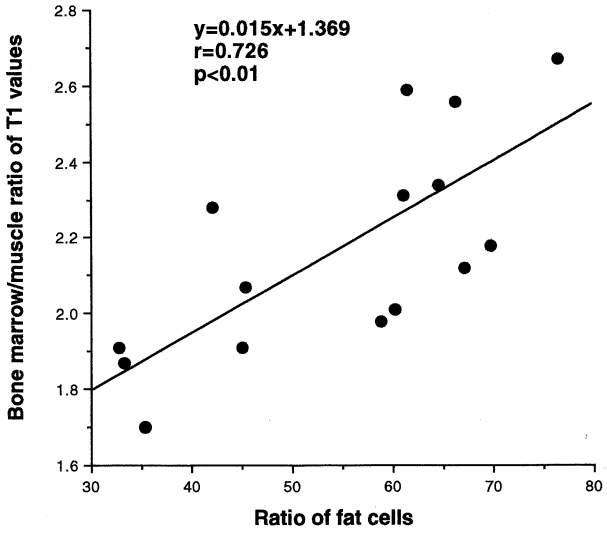

Fig. 3. Correlation of the bone marrow/muscle ratio for the T1-weighted images and the ratio of fat cells in the free bone graft

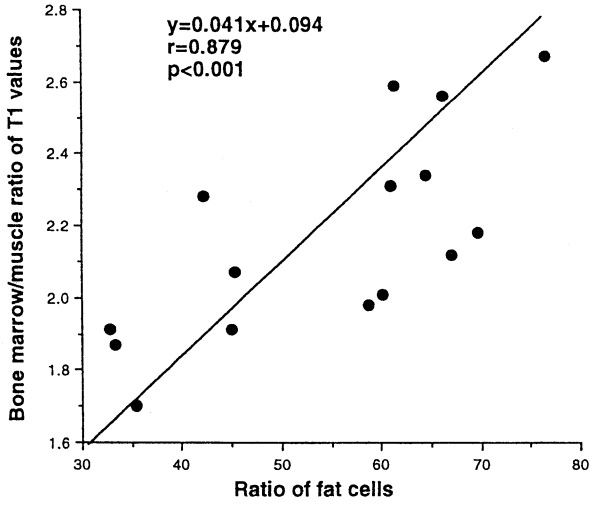

Fig. 4. Correlation of the bone marrow/muscle ratio for the T1-weighted images and the ratio of fat cells in the vascularized bone graft

DISCUSSION

T1-weighted images of femoral head necrosis show a high signal intensity in normal regions due to the abundance of fat cells, and a low signal intensity in necrotic regions.

In this study, the bone marrow/muscle ratio in T1-weighted images showed a correlation with the ratio of fat cells. The bone marrow/muscle ratio of T1 values for the free bone graft was higher than that for the vascularized bone graft during the early period. These results indicate that MR images do not directly represent necrosis. The intensity of the signal of T1-weighted images is assumed to indicate a decrease in the ratio of fat cells in a necrotic area, but not the necrosis itself. Therefore, a necrotic area will show a high signal intensity if fat cells are present, but a low signal intensity with the ingrowth of granulation tissue or the appearance of new bone.

Consequently, our results support the clinical findings of Takatori *et al*[2] The signal intensity of the MR images was low in the area of femoral head necrosis where fibrovascular tissue was present. On the other hand, the necrotic marrow without revascularization showed high signal intensity.

According to Mizuta and Yamasaki,[3] in malignant tumor tissues the reduction in the lipid observed ^{1}H-NMR spectra could be considered as a factor of elevated relaxation times. Therefore, the results suggest that loss of triglyceride esters causes the decrease in signal intensity.

The T1-weighted images of the free grafted bone initially showed a high signal intensity due to the high ratio of fat cells secondary to necrosis of cell components. However, the ratio of fat components decreased as creeping substitution occurred with ingrowth of granulation tissue and new bone formation, resulting in a low signal intensity. Creeping substitution of bone was thought to be complete when the intensity of the signal became stable.

The results of this study suggest that T1-weighted images can demonstrate the course from osteonecrosis to creeping substitution of bone.

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