The difference of calcium deficient hydroxyapatite-induced osteoinduction between dog and rat

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Introduction: There have been some reports that various types of hydroxyapatite, other calcium phosphate-based biomaterials, and porous titanium processed with alkali-heat treatment, had osteoinductivity in the muscles of dogs, sheep, pigs, and primates.

The mechanism of material-induced osteoinduction has not been well understood. In particular, little has not been known about the reason why osteoinduction rarely happen in the muscles of rodents such as rabbits, rats, and mice.

In the present study, we implanted porous calcium deficient hydroxyapatite (CDHA) into the muscles of dogs and rats, and observed the difference of cellular events between the species.

Materials and Methods: Porous CDHA blocks (Ca/P, 1.61; porosity, 70-78%; average pore size, 300um; φ 4 × 4mm cylinder) were supplied by Pentax Co., LTD. These porous CDHA blocks were implanted into the dorsal muscle of five beagle dogs and five SD rats, for periods of 1, 2, 3, 4 or 6 weeks.

After the implants were extracted, some were immersed in 4% paraformaldehyde (PFA) for 2days and decalcified with 20% EDTA-4Na solution for 3weeks in cold room, and then dehydrated with a graded series of ethanol treatments before being embedded in paraffin. Other implants were immersed in 2.5% glutaraldehyde in 0.1M phosphate buffer for 24hours and decalcified with 20% EDTA-4Na solution for 3weeks at 4°C, and then immersed in 1% OsO4 in 0.1M phosphate buffer for fixation and dehydrated with a graded series of ethanol and propylene oxide treatments prior to being embedded in epon.

The specimens embedded in paraffin were sectioned to perform hematoxylin and eosin staining, and tartrate-resistant acid phosphatase (TRAP) staining to detect osteoclast-like multinucleated cells.

The specimens embedded in epon were sliced to observe multinucleated cells by transmission electron microscope (TEM). This study was approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University, Japan.

Results: In the specimens extracted from the dogs, newly bone formation was observed on weeks 4 (Fig. 1A) and 6, whereas in the rats, no bone formation was detected within 6 weeks in all implants (Fig. 1B).

In the dogs, some TRAP positive multinucleated cells were observed in the peripheral region of the implant on weeks 1 and 2. After week 3, a large number of TRAP positive cells were detected in all regions of the implant (Fig. 2A). In the rats, a small number of TRAP positive cells were observed in only the peripheral region of implant on all weeks (Fig. 2B).

According to the TEM observation, multinucleated cells in the specimens extracted from the dogs, showed the polar localization of nuclei and ruffled border-like structures, which were characteristic of osteoclasts (Fig. 3A). However, in the specimens extracted from the rats, they showed no osteoclast-like structure (Fig. 3B).

Discussion: In the present study, osteoinduction was detected in CDHA extracted from the dogs, but was not from the rats. Additionally, the obvious differences in morphology of the multinucleated cells were detected between dogs and rats.

It has been reported that β-TCP implantation in the extra-osseous site of dog shows early appearance and proliferation of osteoclast-like multinucleated cells, and they may play a key role in osteoinduction.

Based on these results, it was suggested that osteoclast-like multinucleated cell is one of the key factors to have osteoinductivity.