The Importance of Osteoclast-like Multinucleated Cells in Material-Induced Osteoinduction

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Introduction
There are some reports suggesting that various types of hydroxyapatite [1], other calcium phosphate-based biomaterials [2], and porous titanium processed with alkali-heat treatment [3], could result in osteoinductivity in the muscles of dogs, sheep, pigs, and primates.

The mechanism of material-induced osteoinduction is not well understood. In particular, it is unclear why osteoinduction rarely occurs in the muscles of rodents such as rabbits, rats, and mice. It has been reported that β-tricalciumphosphate (B-TCP) implantation in the extraosseous site of dog results in the early appearance and proliferation of osteoclast-like multinucleated cells, and they may play a key role in osteoinduction [4].

In the present study, we implanted porous calcium-deficient hydroxyapatite (CDHA) into the muscles of dogs and rats, and observed the difference in cellular events between the species with particular emphasis on osteoclast-like multinucleated cells.

Materials and Methods
Porous CDHA blocks (Ca/P, 1.61; porosity, 70–78%; average pore size, 300μm; 4 × 4 mm cylinder) were supplied by Pentax Co., LTD. These CDHA were implanted into the dorsal muscles of 9 beagle dogs and 18 SD rats for a period of 1–6 weeks. There were 10 and 5 blocks implanted in each dog and rat, respectively. After the CDHA were extracted, some were embedded in paraffin and others were in epon.

The specimens embedded in paraffin were sectioned for hematoxylin and eosin (HE) staining and tartrate-resistant acid phosphatase (TRAP) staining to detect the osteoclast-like multinucleated cells. The specimens embedded in epon were sliced to observe the multinucleated cells by transmission electron microscopy (TEM).

The CDHA harvested from dogs and rats each week was crushed and the total RNA isolated. Thereafter, some osteogenic factors were detected by reverse transcription-polymerase chain reaction (RT-PCR).

Results
In the specimens extracted from dogs, new bone formation was observed after 4 weeks (Fig. 1A), whereas in rats no bone formation was detected in the 6-week period in any implants (Fig. 1B).

Fig. 1 HE staining of the paraffin section. (A) Dog sample at week 4. New bone formation (arrows) was observed. (B) Rat sample at week 6. No bone formation was observed. (Bar = 100 μm, ×: material)

In dogs, a large number of TRAP-positive cells were detected in all regions of the implants after 2 weeks (Fig 2A). However, in rats, a small number of TRAP-positive cells were observed only in the peripheral regions of the implants throughout 6 weeks (Fig 2B).

Fig. 2 TRAP staining of the paraffin section. (A) Dog sample at week 2. A large number of TRAP-positive cells (arrow heads) were observed in all regions of the implant. (B) Rat sample at week 2. Only a small number of TRAP-positive cells (arrow heads) were observed in the peripheral region of the implant. (Bar = 100 μm, ×: material)

According to TEM observation, multinucleated cells in the specimens extracted from dogs after 2 weeks, showed the polar localization of nuclei and ruffled border-like structures that are characteristic of osteoclasts (Fig. 3A). However, in the specimens extracted from rats, multinucleated cells with huge and round cytoplasm possessing no osteoclast-like structures were observed (Fig. 3B).

RT-PCR analysis revealed that the intensity of cathepsin K was higher in dogs than in rats. Alkaline phosphatase (ALP) was strongly detected in dogs after 4 weeks, whereas it was sparsely expressed in rats throughout the 6 weeks. The receptor activator of nuclear factor kappaB (RANK), its ligand RANKL and osteopontin (OPN) were detected both in dogs and rats (Fig. 4).

Fig. 3 TEM image of a multinucleated cell (×1500). (A) Dog sample at week 2. Ruffled border-like structures (arrow) were detected. (B) Rat sample at week 2. The multinucleated cell showed no osteoclast-like structure. (×: material)

Fig. 4 RT-PCR using total RNA isolated from CDHA. ALP was strongly detected in the dog sample after 4 weeks. The intensity of cathepsin K was higher in dogs than that in rats at each week.

Discussion
In our study, osteoinduction was detected in CDHA extracted from dogs after 4 weeks but not in that from rats in 6-week period. It was indicated that the inductivity of CDHA was dependant on the animal species as well as implantable materials that have been reported thus far [2]. The TRAP-positive cells detected in the CDHA from dogs were much larger than those detected in CDHA from rats. Additionally, obvious differences in the morphology of the multinucleated cells from dogs and rats were detected. The level of cathepsin K, an osteoclast marker, was higher in dogs than in rats as detected as by RT-PCR. On the basis of these results, we concluded that in the CDHA from dogs, TRAP-positive multinucleated cells might differentiate into osteoclast-like cells in advance of the bone formation. However in rats, the multinucleated cells did not possess TRAP activities, probably because they could not differentiate into osteoclast-like cells. Since ALP, an osteoblast marker, was strongly detected after 4 weeks in the CDHA from dogs by RT-PCR, it is thought that osteoclast-like multinucleated cells emerge before the differentiation of osteoblasts. Moreover, RANK and RANKL are detected both in dogs and rats. Therefore, the differentiation of these osteoclast-like cells may be due to factors other than the RANK-RANKL signals.

Based on these results and hypotheses, it was suggested that the appearance of TRAP-positive osteoclast-like cells at an early stage may be one of the key factors determining whether or not a species possesses osteoinductive ability.

References