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Tracing process of β-TCP ceramics in vivo with ^{45}Ca①

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Abstract: The metabolic way of calcium ions which was released due to in vivo degradation of porous β-TCP ceramics was studied by using the radioisotope ^{45}Ca as tracer. The result shows that some of the calcium ions enter blood and take part in the circulation. These calcium ions distribute into organs and tissues (such as liver, kidney, brain, heart, lung, spleen and stomach) and participate in the metabolisms of body. There is neither the accumulation of calcium ions, nor the lesion or pathologic calcification of the organs and tissues. Some of the calcium ions that enter the near-end femur, ulna and skull are reused by bony tissue to take part in both local mineralization processes during bone healing, or are stored in calcium pool which can participate in the whole body circulating. In the cyclical process, other calcium ions are excreted with urine and feces through kidney and liver. It is indicated that the degradation products of β-TCP ceramics can take part in the physiological metabolic process of normal bone and tissue.

Key words: β-TCP ceramics; ^{45}Ca tracing; metabolism; mineralization

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1 INTRODUCTION

For a long time, bone substance losing due to congenital, tumorous or traumatic has been treated with bone grafts. In order to avoid the drawbacks of autografts (blood loss, haematoma, pain, risk of infection) or heterografts (rejection, infection, viral risk), various synthetic bone substitutes are now being proposed, in which, calcium-phosphate ceramics (CPC) appears to be suitable, since their chemical composition is very close to the mineral phase of natural bone. These materials have been proved to be well biocompatible, non-inflammatory and non-variable[1, 2]. Being implanted in osseous sites, these materials can directly contact with bony tissue without fibrous interlayer[3]. The most typical CPC is β-tricalcium phosphate (β-TCP) ceramic that exhibits excellent biodegradable property. It can serve as bracket for new bone formation and play the role of osteoconduction[4, 5]. But it is still not known whether calcium ions involved in the mineralization process of new-formed extracellular bone matrixes are provided by the blood flow or by released ions from the resorbing implant. The metabolic way of the degradation products is also not clarified[6, 7]. β-TCP ceramic consists of the elements of calcium, phosphate and oxygen, which have multiple isotopes. As far as half-life, radioactivity intension and ray energy are concerned, the calcium is more suitable than phosphate and oxygen[8]. The method of atom tracing was used in this study. Calcium has various isotopes, in which the half-life of ^{41}Ca is 8×10^4 a, the half-life of ^{45}Ca is 165 d, and the half-life of ^{47}Ca is 45 d. The results of previous animal experiments showed that β-TCP ceramics began to degrade after being implanted in vivo, and almost all of materials degraded after 20 weeks. Hence, ^{45}Ca is comparatively an appropriate tracer. The β-TCP ceramics labeled by ^{45}Ca was placed in the femoral condyle of rabbits. The excreta, organ and tissue of animals and the surplus materials were collected at intervals after implantation, and then the radioactivity was tested. Sequentially, the distribution, metabolism and reuse process of the degraded products were estimated.

2 MATERIALS AND METHODS

2.1 Materials preparation and property

In order to obtain porous β-TCP ceramics, high-purity β-TCP micro-powder was mixed with pore-forming material and high-temperature binder which chiefly consisted of CaO and P_2O_5, then foamed through the rosin foaming method and sintered at 850 ℃ for 2 h. The porous β-TCP ceramics has 50% porosity and exhibits interconnecting pores with a mean pore size of 240－510 μm. The β-TCP ceram-

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ics, with a density of 1.05 – 2.00 g/cm^3 exhibits compression strength of 15 – 30 MPa. The sintered β-TCP structure was formed by neck connecting at grain boundaries. The grain size is about 2 – 3 μm. X-ray results show, and that the main crystal phase of the ceramic is β-$Ca_3(PO_4)_2$, containing a few other calcium phosphate crystal phases and amorphous phase. Cylindrical implants of 8 mm in height and 5 mm in diameter were made and sterilized by high-pressure steam.

2.2 Preparation of ^{45}Ca labeled β-TCP

Samples of porous β-TCP ceramics were made radioactive by thermal neutron bombardment, which was produced by the nuclear reactor of the Atomic Energy Scientific Research Institute of China. The radioactivity of per gram material was 10.12 MBq before implantation.

2.3 Implantation experiments

Ten New Zealand white rabbits in both sexes (provided by Animal Experiment Center of Hubei Province), weighing 2 – 2.5 kg, were divided into five groups according to different stages of implantation. The animals were subjected to a standard operative procedure in the areas of both condyle femurs under general anesthesia. Bone defects of 5 mm in diameter and 8 mm in height in the tibiae condyle for implanting ceramics samples were created at low speed under an asepsis condition. The cavity orientation including spongy and compact bone was perpendicular to the longitudinal and sagittal axis of the tibia. After being washed and cleaned with normal saline, the cylindrical β-TCP ceramics were press-fit inserted into the defects, and each condyle received an implant. The cylinder was lower than the surface of bony tissue. After installation, each implant was covered with its periosteal flap, and then the incision was stratified closed. Each animal was maintained in a separate cage and allowed full weight-bearing.

2.4 Radioactivity testing

After 2, 4, 8, 12 and 20 weeks, the animals were sacrificed with CO_2 asphyxiating. The whole implanted material, together with organ and tissue of animals such as near-end femur, ulna, skull, liver, kidney, brain, heart, lung and stomach were taken out, and then the radioactivity was tested respectively. 0.25 g tissue of each organ and the whole implant were respectively placed in the ground alimsntary test-tube with stopper. Each tube had 2 – 3 mL Perie's liquor, which was saturated with magnesium nitrate(0.5 – 0.6 g/L), and was made of 60% nitric acid and one third of perchloric acid. The samples were water-bathed for 1 – 2 d at 100 ℃, and evaporated to dryness in the balneum arenae at 280 ℃ after being digested completely. After slight cooling of the samples, 1 mL glycerine (100 ℃) and 6mL ethanol solution of N-N-dimethylformamide(volume ratio 1∶3) were dissolved. The samples were displaced into the scintillation cup, in which 10 mL TPP3 scintillation liquor was added. The cycle time per minute (cpm) was measured using the scintillation counter (FJ21084, double canal and combination, China) after mixing uniformly. The efficiency curve was plotted and revised by using the external standard method. The decay time per minute(d) values were calculated referring to the formula of $d = \frac{c}{E}$ (E is efficiency). The decay of radioactivity was adjusted referring to the formula of $A_0 = \frac{A}{1 - \frac{1}{2}(\frac{t_i}{t_p})}$. The formula of $1 - \frac{(\text{Being- implanted- material})}{(\text{Not- being- implanted- material})} \times 100\%$ was used to count the d value of implantation material. Consequently, the reduction rate of radioactivity that could reflect the degradation rate of material was worked out.

The blood, urine and feces of the rabbits were respectively collected and the radioactivity was tested at 3, 6, 10, 16, 30, 77, 107, 126 and 158 d. The decay time of nucleus per minute was shown as d.

2.5 Autoradiograph of liver and kidney tissue

At 1 and 3 months the liver and kidney tissue of animals were excised, the frozen sections were prepared. The sections (6 μm) were placed on microscope slide, then were dried in a desiccator, in which P_2O_5 was used for 48 h. The sections were fixed with anhydrous ethanol vapour, smeared by using maskant of 5% collodion before being shifted to darkroom. The emulsoid (Nucleus-4) was uniformly spread on the tissue slices. The slices were exposed, developed and fixed after natural drying, stained with HE and observed by optical microscope.

3 RESLUTS

3.1 Radioactivity of ^{45}Ca in organic tissue

Radioactivity could be tested in the organic tissue such as liver, kidney, brain, heart, spleen, stomach and lung 2 weeks after implantation. This showed that calcium ions, which were degraded from the implanted materials, were metabolized in various organs by force of blood circulation. The radioactivity variation in different organic tissue during 4, 8 and 12 weeks was not significant. After 20 weeks, the ra-

dioactivity in organic tissues was lower than that of 2 weeks, which indicated that the calcium ions of degradation product were not collected in all of organs (Figs. 1 and 2).

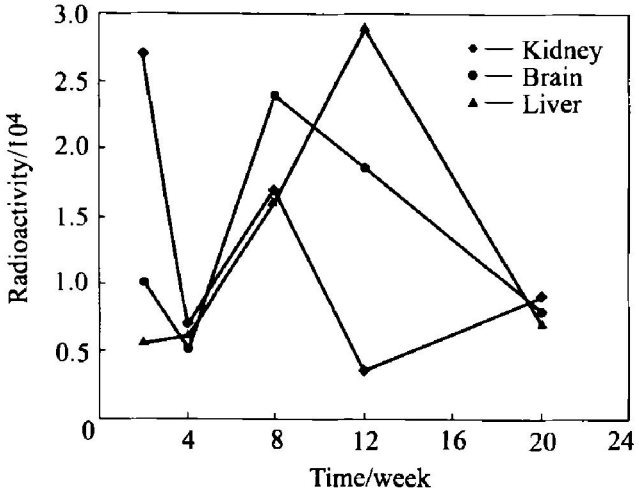

Fig. 1 RA in tissue of kidney, brain and liver

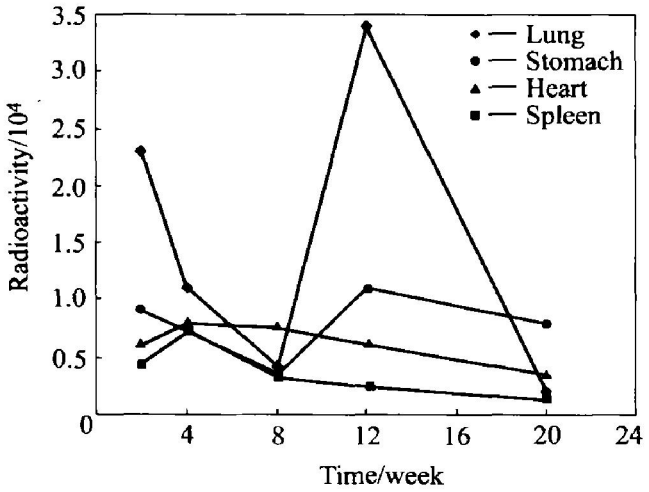

Fig. 2 RA in tissue of lung, stomach, heart and spleen

The radioactivity of calcium in the tissue of near-end femur, ulna and skull was tested at 2 weeks. But the radioactivity values in the near-end femur and the skull were lower than those in all of organs. The radioactivity gradually increased with time. The radioactivity in skull arrived to the maximum peak at 8 weeks. The radioactivity values in the near-end femur and the skull were tenfold or tens-fold higher at 8 weeks than that at 2 weeks. The value in bone tissue was far higher than those in other organs. Moreover, the radioactivity in the near-end femur and skull was higher than the ones in ulna after 12 weeks(Fig. 3). It is shown that the calcium ions of degradation product were collected in bony tissue.

3.2 Radioactivity of ^{45}Ca in blood and excreta

^{45}Ca could be tested in blood, urine and feces 3 d after implantation. Their radioactivity generally increased with time. This is indicated that the degrada

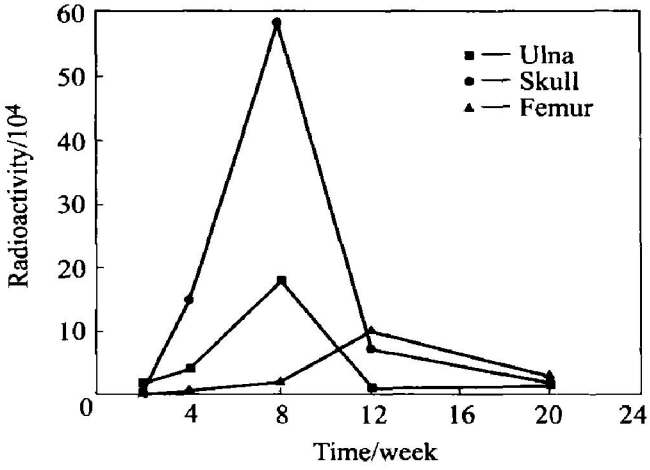

Fig. 3 RA in different bone tissues

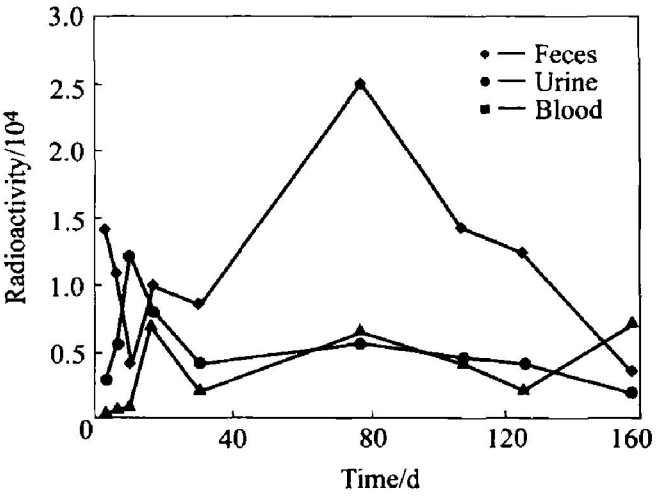

Fig. 4 RA in feces, urine and blood

tion rate of implants increased, which resulted in the increasing of concentration of calcium ions. Around 3 months, the radioactivity of ^{45}Ca reached the maximal value in blood and feces, and the radioactivity in urine was lower than that at 10 and 16 d. But it was higher than that at 2 and 4 months. This showed that the degradation rate was the maximum in this period. The implants still degraded 3 months after implantation, but the degradation rate was relatively reduced because some of implants have been degraded.

3.3 Radioactivity of ^{45}Ca in implants

The radioactivity in the implants was gradually reduced with time. Compared to the material before implantation, the reduction rate of radioactivity was 5.90% at 4 weeks, 32.5% at 8 weeks, 45.67% at 12 weeks and 58.16% at 20 weeks, respectively. This indicated that β-TCP ceramics degraded after implantation.

3.4 Autoradiograph of liver and kidney tissue

A few of silver particles were found to distribute in extracellular space and blood vessel of liver and kid-

ney, ductuli hepaticus communis or urimferous tubules. This reflected the distribution of calcium in tissues after degradation of β-TCP. The number and distribution of silver particles at 12 weeks were the same as that at 4 weeks. The structures of liver and kidney tissue were normal, without damage and calcification (Figs. 5 and 6).

Fig. 5 Tissue structure of liver and distribution of silver grain 12 weeks after implantation

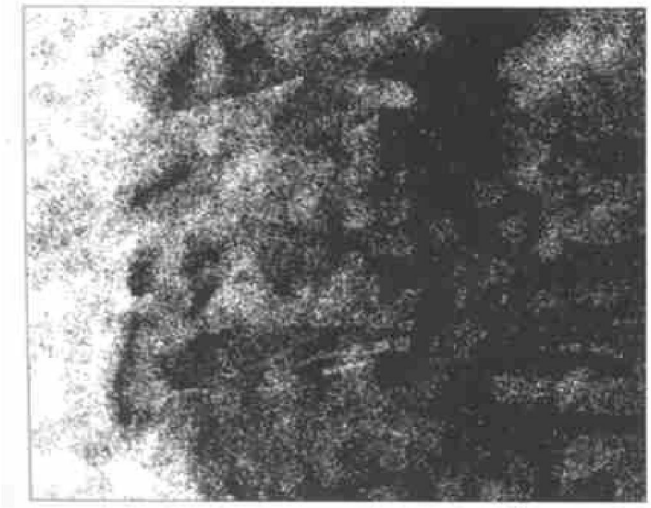

Fig. 6 Tissue structure of kidney and distribution of silver grain 12 weeks after implantation

4 DISCUSSION

The isotopic tracing is extensively used in biologic research because it has high sensitivity, simple operation and is almost close to normal physiologic biochemistry condition of living beings. In this study, β-TCP ceramics that were made radioactive by nuclear activation were placed in the femoral condyle of rabbits and monitored for 20 weeks. By force of the technique of isotopic tracing, the degradation process of β-TCP ceramics and the metabolism process of the degradation products in vivo were investigated scientifically and dynamically by testing the radioactivity of ^{45}Ca. The porous β-TCP ceramics used in this experiment consisted of β-$Ca_3(PO_4)_2$ and calcium phosphate soluble glasses. Its main components, which resemble the mineral of bone matrix, are CaO and P_2O_5. As shown in Figs. 1 and 2, the radioactivity in organic tissue firstly reduced, and then increased gradually. Moreover, ^{45}Ca was found in blood, urine and feces in a short time. This proved that calcium ions have already released from the material due to degradation. The initial degradation took place on the surface and in the pore of material. It began with the dissolution of the binder. The radioactivity in implants gradually reduced with the implantation time. The reduction rate was 58.16% at 20 weeks. As an important index of degradation observation, the reduction rate of radioactivity reflected that β-TCP ceramic evidently degraded, although it incompletely corresponded with the degradation rate of material[9].

In our research, we found that β-TCP ceramics degraded in vivo in two ways: the dissolution process in body fluid and the degradation process mediated by cells. The degradation products were calcium and phosphate ions[10]. The above results showed that the calcium ions released from the materials due to degradation entered blood and took part in the circulation immediately. They were distributed into organs and tissues to participate in the metabolism. Some of the calcium ions were excreted with urine and feces through kidney and liver. The radioactivity of ^{45}Ca was continuously tested in urine and feces after implantation. This indicated that the excretion was going on in succession as the material continually degraded. After 3 months, the radioactivity of ^{45}Ca in blood, urine and feces did not increase continuously but reduced gradually. This showed that the metabolism of ^{45}Ca was not an amount of accumulation, but only a procedure. Therefore, the degradation products of β-TCP ceramics took part in circulation and metabolism by force of blood, and were excreted with urine and feces. There was no forming of accumulation.

The distribution of calcium ions that produced by degradation was homogeneous in organs and tissue. More calcium ions entered organs such as liver and kidney due to the great amount of blood flow, so the calcium concentration was higher in these organs. The results of autoradiograph showed that calcium ions were generally in the blood vessel, extracellular enzyme and tissue space. The calcification and structural damage of organs were not observed by histology. The radioactivity in organs reduced after 20 weeks. This testified that the degradation products were not accumulated. On the contrary, the radioactivity of ^{45}Ca in bony tissue, especially in skull, increased rapidly with time. The ability of new bone formation was most vigorous in bony tissue, which is far higher than that in other organs. This indicated that most of the calcium ions degraded from β-TCP

ceramics were stored in the calcium-pool of body and reused by bony tissue to form new bone. Metsger et al[11] and Irrigaray et al[12] found that the calcium ions that degraded from the calcium phosphate ceramics implanted in bone were precipitated in the implantation region and reused by new bone, but were not used by the mature host bone tissue.

Therefore it could be seen that not all of the calcium ions released from materials took part in the new bone formation. Some calcium ions partook in local mineralization process, some ones went into the circulation and participated in the circulation of calcium pool, and other ones participated in the metabolism of body and discharged with the excretion. The degradable calcium phosphate materials that were used as bone substitutes represent a store, which was probably involved in both local mineralization process during bone healing and circulation of calcium pool, just like physiological bone mineral. This showed that the lifeless degradable calcium phosphate ceramics could be incorporated in the organic tissue activity by force of degradation —— the normal physiologic metabolic process.

5 CONCLUSIONS

The calcium ions that released from β-TCP ceramic enter the blood immediately after implantation. They distribute in all organs and take part in the metabolism of body. Some ones are excreted with urine and feces. There are no damages of organic tissue and pathologic calcification. Other ones are stored in the calcium pool of body, and are used to take part in the local mineralization of new bone in implanted area or the metabolism of host bony tissue, then constitute a part of body.

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