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Bone Marrow Autograft Associated to Macroporous Biphasic Calcium Phosphate for Bone Substitution in an Animal Model of Sequels of Radiotherapy

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Abstract. Bone invasion is common in case of Squamous Cell Carcinomas (SCC) of the upper aero-digestive tract. Radiotherapy is required in addition to large surgical tumor removal. This treatment usually generates irreversible injuries on the reparation properties of the tissues, especially on bone. The quality of life of patients undergoing major surgery and radiotherapy in maxillary and mandible areas is reduced, but could be improved by bone reconstruction. The aim of this study was to evaluate the bone reconstruction possibilities by Macroporous Biphasic Calcium-Phosphate (MBCP™). The MBCP substitute was evaluated as granules and associated to autologous bone marrow (BM) graft in irradiated areas, in an inbreeding rodent model. Radiation sequels were created on inferior members of half of the rats. 3 weeks later, 3-mm osseous defects were created on each animal. The inbreeding model allows BM to be grafted without graft-versus-host reaction. Defects were filled either with MBCP alone, BM alone or a mixture of MBCP and BM. Six weeks after implantation, animals were sacrificed: bone repair and ceramic degradation were evaluated by qualitative and quantitative study. Results showed that bioceramics were well osteointegrated. Filling the defects with BM alone showed a significant increased of newly-formed bone formation but only after irradiation, whereas filling defects with MBCP alone increased new-bone formation only without previous irradiation. Associating MBCP to BM provided the best new-bone formation rates after irradiation. Degradation of the ceramic was the most important in case of BM grafting. This study demonstrated that BM added to MBCP constitute an appropriate material to be considered in case of bone defect occurring in irradiated tissue, and could be foreseen for use after bone removal for oncologic obligations.

Introduction

Calcium-phosphate materials are currently used in surgery as bone-bonding materials [1-3]. Irradiation produces irreversible effects on normal tissues, involving damages on their reparation properties. Nevertheless quality of life of patients who undergo radiotherapy could be improved by bone reconstructions [4]. Until now, bone substitution by MBCP has only been evaluated, with good results, prior to irradiation delivery, but never in a irradiation sequels model. The purpose of this study was to create an animal model of sequels of bone radiation therapy, and to determine the behavior and benefits of bone substitutes associating MBCP Ceramics and BM grafts. The amount of newly-formed bone and of ceramic degradation were evaluated and compared to control defects. Results were discussed according to implantation procedure: BM graft alone, BCP granules alone or BCP granules and BM mixture.

Materials and Methods

14 inbreeding Lewis 1A adult rats were included in the study. 2 of them were specially designed as BM graft donors. 6 of the 12 animals were randomized to receive an external irradiation and the other 6 animals were kept free of irradiation. The radiations were delivered by photons of ^{60}Co under a Therathon 780 (Atomic Energy, Canada). A single dose of 15 Grays localized on the inferior members was delivered under intra-peritoneal anesthesia obtained by 10 mg sodium thiopenthal (Rhône-Mérieux, Lyon, France).

3 weeks after the external radiation delivery, 4 osseous defects (2 tibial and 2 femoral) were performed on the 12 animals (irradiated and non-irradiated) under general anesthesia. BM was obtained from the donor animals under general anesthesia before those animals were sacrificed. Femurs from BM donor animals were removed and the marrows were collected after flushing out 0.5 ml of physiological serum through the bone shaft sections. A cytological myelographic analysis was performed on the BM, which were then immediately and aseptically placed in heparinated tubes.

Critical-sized defects were calibrated at 3-mm in diameter, then randomly divided into 4 groups: kept empty as control, or filled with either MBCP granules alone (mean diameter of 700 µm), filled with BM graft alone or with a mixture of BM and MBCP.

6 weeks after implantation, animal were sacrificed by injection of an overdose of sodium thiopenthal. The implanted and control locations were fixed in 4% paraformaldehyde phosphate-buffered saline (Seromed Berlin, Germany), dehydrated, then infiltrated and embedded in glycol-methacrylate for histologic evaluation. Horizontal 7-µm sections perpendicular to the long axis of the bone were obtained without decalcification, at the largest diameter of tibial or femoral bone defects, using a Leica apparatus (Nußloch, Germany). Solochrome-cyanine R and Movat's pentachrome histomorphochemical stainings sections were studied under a light microscope.

GMA embedded bone specimens were sanded then carbon-coated on a JVG N1 (Jeol, Tokyo, Japan) and SEM studies were performed with backscattered electrons at 15 kV in conjunction with image analysis. Quantitation of newly-formed bone and resorbed/degraded MBCP was determined using a semi-automatic QC 500 image analyser (Leica, Japa). Image analyser determined the respective areas of bone marrow, ceramic and newly-formed bone, according to the magnification. Calculations were performed inside the implanted defect area and results were expressed as the relative percentage ± SD of newly-formed bone and of the remaining MBCP ± SD for each implantation condition.

Bone ingrowth and MBCP degradation/resorption were statistically compared using analysis of variance (Fisher's procedure, ANOVA, significance level $p<0.05$).

Results

Surgical procedure and external radiation delivery were completed without major complications. There was neither infection, nor exteriorization of the implants after surgical implantation or during irradiations. The usual secondary effects of external radiotherapy were noticed: reversible dermatitis, asthenia and weight loss.

Cytological myelographic analysis showed that BM contained quantitative (51.10^6 cells/mm^3) and qualitative physiological lineage cells (i.e. myeloblastic, myelocytic, proerythroblastic, erythroblastic, megacariocytic, lymphocytes, plasmocytes and monocytes), without any blood recover.

Histological studies revealed the absence of newly-formed bone in control defects after irradiation. In case of implantation of MBCP alone after irradiation, no fibrous interposition around the granules was noticed, but new-bone formation areas were thin. New-bone was arising from the periphery of implants, between countersunk cortical banks, and decreased gradually toward the center of the defects. In contrast, MBCP and BM mixture provided woven bone, not only in the border of implanted defects but also in the deep spaces. Marrow spaces were composed of inflammatory cells and rich new vascular structures.

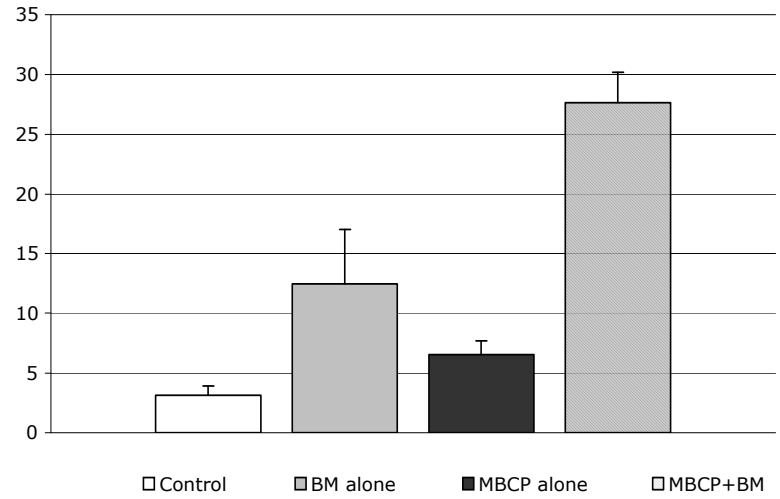

Fig. 1, New-bone formation in irradiated bone defects. (*p=0.0028) between BM and control; #,•p<0.0001 between MBCP - BM mixture and control and MBCP alone).

Quantitative image analysis: results are reported in figure. Newly formed bone was significantly increased by MBCP implantation in non irradiated bone compared to control defects (p=0.003) and compared to BM alone (p=0.043). BM implantation alone in defects did not significantly increased bone formation compared to control defects in absence of irradiation. After irradiation, bone ingrowth's was increased after BM implantation alone (p=0.0028) and after MBCP associated to BM (p<0.0001) compared to control defects. Addition of BM to MBCP implants in defects showed to provide significant improve of bone formation compared to BM alone (p<0.0001). In opposition to non irradiated defects, MBCP alone compared to control did not improve the new bone formation.

Degradation of the ceramic was significantly increased when MBCP was combined with BM in irradiated areas (p=0.017), although degradation was not modified by the presence of radiation before defect was created.

Discussion

The results of this study showed that very little bone formations were observed in irradiated bone defects, always in the border of implanted areas in case of MBCP implantation alone. Moreover, MBCP implanted alone was unable to increase bone ingrowth's compared with control. Nevertheless, the granules had very close contact to bone, without fibrosis encapsulation indicating persistence of integration properties of the material after irradiation. BM implanted alone was accompanied by an increase of bone formation only in irradiated areas, but not in normal tissue, suggesting that mesenchymal stem cells decrease after irradiation in bone. In non-irradiated tissues, the communication of medullar bone with the bone defect can explain the absence of increase of bone formation rate after BM graft, as BM was directly present in the defects and sufficient. The number of mesenchymal stem cells remaining after irradiation could be a limitation factor for bone substitution [5], by contrast with normal bone. After implantation of MBCP and BM mixture, new-bone formation was observed not only in the border of the implant, but also deeply inside the granules. This result suggests that BM in contact to MBCP after irradiation is able to induce a new-bone formation distantly to residual bone [6]. The amount of inflammatory marrow and new vascular formations that were observed put forward that the grafted BM was able to induce marrow expansion inside irradiated bone defect. With MBCP and BM associated, bone ingrowth was also significantly increased, whereas not with MBCP alone indicating that stem cells added to MBCP during BM graft were able to differentiate into osseous progenitors [7]. At the same time BM graft provided functional cells for

ceramic degradation. The amounts of newly formed bone obtained indicate that bone substitution can also be recovered after radiation therapy provided that autologous BM is added to MBCP. Thus BM and MBCP mixture constitutes a new perspective for bone substitution in irradiation territories.

Conclusion

BM can be easily obtained and added to MBCP granules for bone substitution in defects occurring in irradiated tissues. The lack of bone healing capacities after radiotherapy, and the observed results obvious the lack of osteoconductive properties of Calcium-Phosphate ceramics in irradiated bone. The decrease of stem cells after irradiation can be prevented by addition of autologous BM with MBCP granules, and the material could be foreseen for use after bone removal for oncologic obligation.

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