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Original Article

Effects of ionizing radiation on proteins in lyophilized or frozen demineralized human bone

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ABSTRACT

Objective: The aim was to study the effects of application of ionizing radiation (gamma and electrons) as sterilizing agents at doses of 15 kGy, 25 kGy and 50 kGy, on lyophilized or frozen demineralized bone tissue for use in transplants.
Methods: Five human femoral diaphyses from different donors of musculoskeletal tissue were demineralized and preserved as lyophilized or frozen at −80 °C. The samples were divided into two groups: non-irradiated (control) and irradiated by means of gamma rays or an electron beam. The bone proteins were extracted and used to determine the concentrations of total protein and BMP 2 and 7.
Results: Decreases in total protein and BMP 2 and 7 concentrations were observed. The decreases in total protein concentrations, in comparison with the respective control groups, were significant in the lyophilized and frozen samples that were irradiated at a dose of 50 kGy of gamma radiation and electron beam, with reductions of more than 30%. Significant decreases in the levels of BMP 2 and 7 were also observed at higher doses and especially through use of the electron beam.
Conclusion: The reductions in the concentrations of total proteins and osteoinductive proteins (BMP 2 and 7) were related to the radiation dose, i.e. they increased with higher doses of ionizing radiation type and the type of bone preservation. The largest reductions in concentrations were observed in the bones irradiated by means of an electron beam and at a dose of 50 kGy. However, this type of radiation and this high dose are not usual practices for sterilization of bone tissue.

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Efeitos da radiação ionizante nas proteínas presentes em ossos humanos desmineralizados, liofilizados ou congelados

Palavras-chave:
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Radiação ionizante
Banco de tecidos
Proteína morfogenética óssea 2
Proteína morfogenética óssea 7

RESUMO

Objetivo: Estudar os efeitos da aplicação das radiações ionizantes (gama e elétrons) como agentes esterilizantes, nas doses de 15 kGy, 25 kGy e 50 kGy, nos tecidos ósseos desmineralizados congelados e liofilizados para uso em transplantes.

Métodos: Cinco diáfises femorais humanas de doadores distintos de tecidos musculoesqueléticos foram desmineralizadas e preservadas como liofilizadas ou congeladas a −80 °C. As amostras foram divididas em grupos não irradiados (controle) e irradiados por raios gama ou feixe de elétrons. As proteínas ósseas foram extraídas e dosadas as concentrações de proteínas totais, BMP 2 e 7.

Resultados: Foi observada diminuição das concentrações de proteínas totais e BMP 2 e 7. A diminuição das concentrações de proteínas totais, quando comparada com o respectivo controle, foi significativa nos grupos de amostras liofilizadas e congeladas e irradiadas na dose de 50 kGy por radiação gama e feixe de elétrons com redução superiores a 30%. A diminuição significativa nas concentrações das BMP 2 e 7 também foi observada nas maiores doses e principalmente por feixe de elétrons.

Conclusão: As reduções nas concentrações das proteínas totais e em proteínas osteoindutoras (BMP 2 e 7) foram relacionadas à dose de radiação, ou seja, aumentam com maiores doses, tipo de radiação ionizante e ao tipo de preservação dos ossos. As maiores reduções das concentrações foram observadas nos ossos irradiados por feixe de elétrons e na dose de 50 kGy. Porém esse tipo de radiação e essa alta dose não são práticas usuais para a esterilização dos tecidos ósseos.

Introduction

There is a growing demand for allogeneic musculoskeletal grafts for orthopedic and dental reconstruction in Brazil. Bone tissue can be highlighted within this, with 21,681 distributions in 2014.

Musculoskeletal tissue banks are organizations that take on the responsibility for selecting donors and obtaining, processing, storing, distributing and performing quality control on tissues.

Bone tissues can be obtained from both living and deceased donors. These musculoskeletal tissues are mainly used in revision arthroplasty procedures on hips or knees, in treating patients with bone tumors, in joint reconstruction for treating ligament injuries, in raising the maxillary sinus in cases of atrophy and in mandibular grafts for placement of dental implants.[1]

The ideal allogeneic material for use as a grafting option should have the following properties: biocompatibility, not causing infection, low immunogenicity, osteoconduction and osteoinduction.

Osteoinduction is an osteogenesis process that promotes recruitment of mesenchymal pluripotent undifferentiated cells and stimulation toward differentiation into bone-forming cells. Bone morphogenic proteins (BMPs) are an important group of glycoproteins extracted from demineralized bone matrix that are responsible for bone induction.[2]

BMPs are classified as a subfamily within the superfamily of transforming growth factor-β (TGF-β). According to the literature, BMPs 2, 4 and 7 have the greatest potential for inducing osteoblastic differentiation from progenitor mesenchymal cells.

Performing a demineralization procedure on cortical bone matrix increases the biological availability of BMPs and makes these grafts osteoconductive.[3] There is a positive correlation between the BMP content that is present in grafts and the degree of osteoconductivity.[4]

There is great concern about ensuring tissue quality and promoting safety among patients who receive homogenous tissues, with regard to transmission of infectious and contagious diseases.

With the aim of eliminating possible contamination, bone tissue donors undergo serological screening and assessment of their histories and social behavior, along with molecular biological tests to detect viral RNA from HIV and HCV, clinical examinations and microbiological control tests. Aseptic techniques are applied during the procedures.[5] However, there is the possibility of contamination with microorganisms during tissue acquisition, processing, preservation and storage.[6]

Many professionals responsible for tissue banks consider that it is important to sterilize biological tissues using an effective method such as ionizing radiation.

Sterilization through ionizing radiation is a method that presents advantages over other methods. It produces only a minimal increase in temperature and does not leave toxic residues, which makes it usable and also a form of final sterilization.[7] However, some authors have concluded that sterilization through ionizing radiation may cause dose-related structural and biological alterations to bone tissues.[5,8]