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Validation of a method for non-invasive in vivo measurement of growth factor release from a local delivery system in bone

Juan J. Delgado [a], Carmen Evora [a], Esther Sánchez [a], Manuel Baro [b], Araceli Delgado [a,*]

[a] *Departamento de Ingeniería Química y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de La Laguna, 38200, La Laguna, Tenerife, Spain*
[b] *Servicio de Traumatología, Hospiten Rambla S.L., Santa Cruz de Tenerife, Spain*

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Abstract

The therapeutic benefit of local administration of growth factors using controlled release systems for bone regeneration is under study. In the present work, a PDGF release profile from chitosan granules administered into bone defect produced in the femur of Wistar rats was obtained using ^{125}I-PDGF as tracer and a probe-type gamma counter with a suitable collimator for detection. The measurement method was validated by the radioactivity values obtained from the isolated bones using a well-type gamma counter. Both the invasive and the non-invasive measurement methods were linear in the analyzed range, with coefficients of variation around 3% and 15%, respectively. A good correlation between the two methods was found for the ^{125}I-PDGF release profile from the chitosan granules. These results confirm that a reliable release profile can be obtained for a drug incorporated into delivery systems for local bone therapy using this non-invasive measurement method.

Keywords: Chitosan granules; Controlled drug delivery; Gamma counting; Radiolabelled growth factor; Validation method

1. Introduction

Administration of growth factors (GF) offers attractive potential solutions for bone defect repair. In fact, the fibrin coagule, obtained from plasma rich in GF (PRGF), is currently used in oral-maxillofacial surgery, considerably enhancing bone regeneration. Its main advantage is the high biocompatibility due to the autogenous source. However, there are important disadvantages, such us having to be prepared in situ, its uncertain and variable content, and the very fast release of GF.

These problems may be overcome by the design and optimization of delivery systems based on bioresorbable and biocompatible materials for local administration of the recombinant human GF involved in bone regeneration. On testing this strategy in vivo by local administration in a delivery system, it has to be considered that GF concentrations in serum are often well below the detection limits of most analytical procedures and endogenous levels could also interfere with the results. For that reason, the most suitable method to characterize GF release profiles may be the use of the corresponding radiolabeled GF as tracer. This method was already adopted in a study [1] applying ^{125}I-labeled GF to monitor the release rate by measuring the radioactivity left in implants which were surgically removed after subcutaneous implantation. Although a viable in vivo procedure, it is invasive, being limited above all by the high number of animals needed. Despite its disadvantages, this procedure is still in use [2,3].

For testing drug delivery systems in bone, laborious surgery is required to create a bone defect in the animals [4–6] and thereafter the bone has to be surgically removed for analysis. Furthermore, at least two or three animals have to be used for each time point sampled to determine the remaining radioactivity and standard deviation. In addition, a subsequent scale-up of the animal model is advisable to obtain more reliable information about the properties and effectiveness of the delivery systems in bone regeneration [7].

Since the number of growth factors involved in bone regeneration and the desired kinetics (duration and rate of GF release) are still unknown, a large number of animal experiments would be needed in order to obtain an optimum GF delivery system for bone regeneration.

* Corresponding author. Tel.: +34 922318507; fax: +34 922318506.
E-mail address: adelgado@ull.es (A. Delgado).

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In the present work, a non-invasive method using ^{125}I-PDGF (^{125}I platelet-derived growth factor) as tracer was developed and validated to obtain in vivo growth factor release profiles from local delivery systems. To this end, the ^{125}I-PDGF was incorporated into chitosan granules and administered to a defined bone defect produced in rat femurs.

2. Materials and methods

2.1. Materials

Chitosan (CHT) Mw 400 kDa (75–85% deacetylated) (Aldrich), tripolyphosphate (TPP) (Sigma), tricalcium phosphate (TCP) (Panreac), recombinant human platelet-derived growth factor-BB (PDGF) (R&D Systems) and radiolabelled PDGF (^{125}I-PDGF) (Amersham).

2.2. PDGF-chitosan granule elaboration

Chitosan granules containing PDGF were prepared by the ionic gelation method. Briefly, 2.5% chitosan (125 mg) solution in 3% acetic acid containing 0.004% PDGF (w/w) and ^{125}I-PDGF as tracer (2 μCi) was dropped into 80 ml of an aqueous solution of TPP 5% (w/v) as a cross-linking agent, under magnetic stirring (800 rpm), at a rate of 0.3 ml/min. For this purpose, a fixed perfusion pump with a 50° slope under an air-stream following the dropping axis was used. The resulting granules were separated by filtration, washed with deionized water and freeze-dried.

2.3. TCP/CHT gel preparation

A 1:1 mixture of TCP/CHT 3% in acetic acid (1%) was homogenized by mechanical stirring at 10,000 rpm (Silverson®LTR4) for 10 min and then freeze-dried. A gel was obtained by reconstituting the freeze-dried mixture in water at a ratio of 75.6 mg/ml.

2.4. In vitro characterization of granules

2.4.1. PDGF loading

PDGF loading was determined by measurement of radioactivity levels in granule aliquots, using a well-type gamma counter (Cobra II®, Packard). The loading efficiency was calculated in relation to the initial amount of radioactivity used.

2.4.2. Granule shape and size

Granule shape was examined by scanning electronic microscopy (SEM) and average size was determined by measuring the longer and shorter diameters of 100 granules using a microscope with an ocular micrometer.

2.5. In vivo studies

The local committee for animal studies of the University of La Laguna had previously approved animal experiments which were carried out in male Wistar rats (250–280 g), purchased from the university animal house. The animals had free access to food and water before and during the experimental period.

2.5.1. Administration of granules

Chitosan granules (5 mg), dispersed in a minimum amount of TCP/CHT gel, were introduced with a small spatula into a bone defect made in the femur of male Wistar rats (Fig. 1). The surgery to produce the bone defect was performed as previously described [4]. Briefly, the rats were anaesthetized intramuscularly with ketamine (75 mg/kg) and xylazine (10 mg/kg) and their right hind legs shaved and disinfected. A vertical external parapatellar incision was made in the knee. Then a dislocation of the patellar tendon and quadriceps was performed to allow access to the femoral condyles. A hole in the intercondylar space was made with a 1.8 mm dental burr to reach the medullar cavity. After granule insertion into the medullar space, the patella and the patellar tendon were relocated and the wound was closed with stitches. The surgical wound was disinfected and IM magnesium metamizol (60 mg/kg) was injected. Animals recovered from anaesthesia were allowed to freely move in their cages.

2.5.2. ^{125}I-PDGF measurement

2.5.2.1. Non-invasive method. Non-invasive ^{125}I-PDGF measurements were carried out using a probe-type gamma counter (Captus®, Nuclear Iberica). The radiation at the measurement site was collimated using a lead shield with a 2 cm diameter cylindrical entrance that focuses on the administration site (Fig. 2). The rat was anaesthetized and the femur positioned perpendicularly to the detector, thus the collimator hole being positioned over the knee for external measurement of the remaining radioactivity. At each time point sampled, five readings were taken and the ^{125}I emission peak (maximum 27 keV) was integrated for the cumulative events registered over 1 min. The highest and lowest values were excluded and means of the other values were considered as the remaining radioactivity at each time point. After recovering from anaesthesia, the animal was allowed moving freely in its cage. Thus, a complete release profile was obtained from each animal.

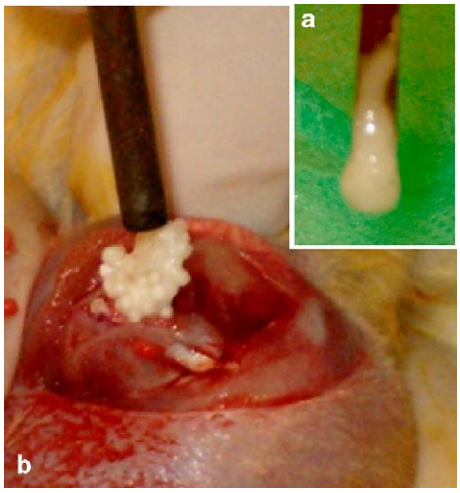

Fig. 1. Pictures of (a) TCP/CHT gel and (b) PDGF-chitosan granules dispersed in the same gel, ready for administration into the femur defect of a rat.