International Journal of Surgery 24 (2015) 120–123

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Original research

Effects of shock wave therapy on glycosaminoglycan expression during bone healing

Paulo Roberto Dias Dos Santos [a,*], Valquiria Pereira De Medeiros [b], João Paulo Freire Martins de Moura [a], Carlos Eduardo da Silveira Franciozi [a], Helena Bonciani Nader [b], Flavio Faloppa [a]

[a] Department of Orthopaedics Surgery, Escola Paulista de Medicina/Universidade Federal de São Paulo, São Paulo, SP, Brazil
[b] Department of Biochemistry, Escola Paulista de Medicina/Universidade Federal de São Paulo, São Paulo, SP, Brazil

HIGHLIGHTS

- Glycosaminoglycans in osteogenesis after shock waves.
- ESWT stimulates chondroitin sulfate and hyaluronic acid.
- Glycosaminoglycans in matrix bone.

ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form
27 August 2015
Accepted 22 September 2015
Available online 30 September 2015

Keywords:
Glycosaminoglycans
High energy shock wave
Osteogenesis
Bone fractures therapy

ABSTRACT

Background: Several cases of delayed bone consolidation have been treated with extracorporeal shock wave therapy (ESWT) to improve bone healing and a key role of the extracellular matrix glycosaminoglycans in osteogenesis has been suggested.
Objective: In this study, we aimed to identify and quantify the amount of sulfated glycosaminoglycans (GAG) and hyaluronic acid (HA) within rat femurs following bone drilling and treatment with shock waves.
Methods: To identify and quantify the sulfated glycosaminoglycans (GAG) and hyaluronic acid (HA) within rat femurs following bone drilling and ESWT, 50 male Wistar rats were evaluated. The animals were divided into two groups, both of which were subjected to bone drilling. One of the groups was treated with ESWT. The rats were sacrificed on the 3rd, 7th, 14th, 21st, and 28th day. GAG presence was analyzed by agarose gel electrophoresis with subsequent densitometry and ELISA.
Results and discussion: The content of sulfated GAGs increased significantly from the 3rd to the 28th day ($p = 0.002$). Chondroitin sulfate was expressed more highly than the other GAGs. HA content increased significantly at the 3rd day in animals treated with ESWT compared to the control group ($p = 0.003$).
Conclusion: ESWT stimulates of sulfated glycosaminoglycans during bone healing and enhanced early expression of HA compared to the control group.
© 2015 IJS Publishing Group Limited. Published by Elsevier Ltd. All rights reserved.

1. Methods

1.1. Bone defect model

All procedures and protocols were performed according to the guidelines of the Institutional Ethics Committee of the Federal University of São Paulo, Brazil and the Ethic's committee approved this study.

Seventy-five femurs from 50 male Wistar rats were evaluated. Rats were 3 months old and had an average weight of 300 g. Animals were housed in a propylene box (5 rats per box) subjected to

List of abbreviations: BMP, bone morphogenic protein; Cetavlon, cetyl trimethylammonium bromide; CS, chondroitin sulfate; ELISA, enzyme-linked immunosorbent assay; GAG, glycosaminoglycan; HA, hyaluronic acid; VEGF, vascular endothelial growth factor.

* Corresponding author. Rua Dr Candido Espinheira 831, ap 51, São Paulo, Brazil.
E-mail address: prds@uol.com.br (P.R. Dias Dos Santos).

http://dx.doi.org/10.1016/j.ijsu.2015.09.065
1743-9191/© 2015 IJS Publishing Group Limited. Published by Elsevier Ltd. All rights reserved.

12 h of light/dark cycles with food and water *ad libitum* and the temperature was controlled (20 ± 2 °C).

Animals were anesthetized by an intraperitoneal injection of ketamine and xylazine at a ratio of 2:1 using 0.2 cc/100 g per animal. Animals were restrained in the prone position, the hind legs were shaved and disinfected with 70% alcohol, and a skin incision, at the thigh, with a scalpel blade no. 15 was performed. The incisions were 3 cm in length and muscle dissection was performed in order to expose the femur. An electric drill, with a 2-mm drill, was used to create a defect in the femoral diaphysis. Drilling was performed until the cortical bone on the opposite side was reached. The wound was sutured using mononylon 4–0 sutures [9].

The animals were divided into two groups. In group I (25 animals) drilling was performed bilaterally and only the left femur (Group IA, 25 femurs) was subjected to ESWT after drilling. The right femur was no subjected to ESWT after drilling (Group IB, 25 femurs). In group II (25 animals, 25 femurs) drilling only was performed in the left femur. Following the procedure, the animals received analgesics and antibiotics for 7 days. Animals were sacrificed, with a CO_2 chamber, on days 3, 7, 14, 21, and 28 (5 animals per day).

The paws were examined daily, and no infection or clinical sign of inflammation were detected. The femurs were removed, fixed in 4% p-formaldehyde in 0.1 M phosphate buffer pH7.2 for 48 h, and decalcified in EDTA (0.7 g/L), tartrate potassium sodium (8 mg/L), hydrochloric acid (99.2 mL/L) and sodium tartrate (0.14 g/L); for 12 h. Subsequently, the fragments were soaked in absolute ethanol. Fragments of 5 mm from each femur containing the lesion area were cut, weighted and submitted to analytical procedure.

1.2. Extracorporeal shock wave therapy application

An electro-hydraulic shock wave generator EVOTRON-Vet® (SwiTech, Switzerland) with 500 pulses and an energy equivalent to 0.13 mJ/mm^2 was used. Only one ESWT session, to the left femur in Group IA, was performed after skin closure [10,11].

1.3. Extraction and quantification of sulfated glycosaminoglycans and hyaluronic acid

Demineralized bone fragments (5 mm) were pulverized using liquid nitrogen. Samples were incubated with 1 mg mL^{-1} papain (Calbiochem, Darmstadt, Germany) in 0.08 M phosphate buffer-cysteine pH 6.5, containing 0.02 M EDTA for 18 h at 60 °C.

Afterwards, peptides and nucleic acid fragments were removed by precipitation with 10% trichloroacetic acid (TCA) at 4 °C. After centrifugation (10 min, 3500g, 4 °C), the supernatant containing GAGs were precipitated by adding two volumes of methanol for 18 h at 4 °C. The precipitate was collected by centrifugation (10 min, 3500g, 4 °C), dried, suspended in 40 μL of distilled water and analyzed for sulfated GAGs and HA contents. Recovery of GAGs extracted from the bones through that method was around 95% [26]. Sulfated GAGs were identified and quantified by agarose gel electrophoresis in 0.05 M 1,3-diaminopropane acetate buffer, pH 9.0. An aliquot of 5 μL of each sample was submitted to electrophoresis for 1 h at 100 V; GAGs were precipitated in the gel with 0.1% Cetavlon (cetyl trimethylammonium bromide) for 2 h at room temperature. The gel was dried and stained with a 0.1% solution of toluidine blue in acetic acid–ethanol–water (0.1:5:4.9, vol/vol). Quantification was carried out by densitometry at 530 nm of the toluidine-blue-stained electrophoretic slide. The extinction coefficients of the GAGs were calculated using standards of chondroitin sulfate (CS), dermatan sulfate and heparan sulfate. Identification of the sulfated GAGs was based on the migration of the compounds compared with those of standards. The identities of GAGs present in the samples were further confirmed by treatment with specific enzymes. Samples of the GAGs isolated from the bones were degraded with chondroitinase AC and chondroitinase ABC (Sigma) in 0.05 M Tris–HCl buffer pH 7.0 at 37 °C. The electrophoresis was performed in triplicate. Results of the absolute amounts of GAGs were expressed per weight of decalcified femur (μg g^{-1}) [12].

HA was quantified by fluorometric noncompetitive enzyme-linked immunosorbent assay (ELISA)-like assay that can detect 2–500 g/L of HA. The ELISA plates had a fixed probe and 100 μl/well of standard HA solutions, at various concentrations (0–500 g/L), were added. The samples were diluted (1:100) in a buffer Tris–HCl 0.05 M, with 1% bovine serum albumine and added to the ELISA plates in triplicate. The plates were incubated at 4 °C for 12 h, followed by three washes with Tris–HCl 0.05 M.

Next, 100 μl of the probe (1 mg/ml) diluted (1:10.000) in the assay buffer was added. The plate was incubated for 2 h on a shaker and washed nine times with a wash buffer. Streptavidin labeled with europium diluted 1:10.000 in assay buffer was added to each well (100 μl; Sigma, Germany). Streptavidin has an affinity for the biotin-conjugated probe. The plate was incubated 30 min on a shaker and washed nine times with Tris–HCl 0.05 M. To release the europium bound to streptavidin (Sigma), an enhancement solution (280 μl/well) was added. The plates were agitated for 5 min and the europium-free plate fluorescence was read on a fluorimeter. The results were expressed in ng/ml [13,14].

1.4. Statistical analyses

Mean values between groups were compared using an Two-way ANOVA [15], followed by Tukey's multiple comparison. A p-value of ≤0.05 was considered to be statistically significant.

2. Results and discussion

2.1. Characterization and quantification of sulfated glycosaminoglycans (Groups IA, IB and II)

The electrophoretic behavior in agarose gel of the glycosaminoglycans extracted from femurs of each experimental condition showed the presence of a single band migrating as chondroitin sulfate.

ESWT leads to an increase in the amounts of CS compared with the non treated femur.

GAG content (ug/g tissue) in Group IA (ESWT group) and Group

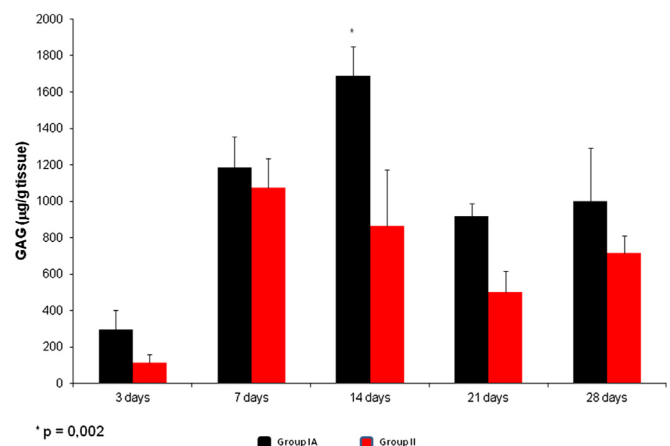

Fig. 1. GAGS Group IA and Group II.

Download English Version:

https://daneshyari.com/en/article/4285764

Download Persian Version:

https://daneshyari.com/article/4285764

Daneshyari.com