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Effects of mechanical loading on the expression of pleiotrophin and its receptor protein tyrosine phosphatase beta/zeta in a rat spinal deformity model

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ABSTRACT

Mechanical loading of the spine is a major causative factor of degenerative changes and causes molecular and structural changes in the intervertebral disc (IVD) and the vertebrae end plate (EP). Pleiotrophin (PTN) is a growth factor with a putative role in bone remodeling through its receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ). The present study investigates the effects of strain on PTN and RPTPβ/ζ protein expression in vivo. Tails of eight weeks old Sprague-Dawley rats were subjected to mechanical loading using a mini Ilizarov external apparatus. Rat tails untreated (control) or after 0 degrees of compression and 10°, 30° and 50° of angulation (groups 0, I, II and III respectively) were studied. PTN and RPTPβ/ζ expression were evaluated using immunohistochemistry and Western blot analysis. In the control group, PTN was mostly expressed by the EP hypertrophic chondrocytes. In groups 0 to II, PTN expression was increased in the chondrocytes of hypertrophic and proliferating zones, as well as in osteocytes and osteoblast-like cells of the ossification zone. In group III, only limited PTN expression was observed in osteocytes. RPTPβ/ζ expression was increased mainly in group 0, but also in group I, in all types of cells. Low intensity RPTPβ/ζ immunostaining was observed in groups II and III. Collectively, PTN and RPTPβ/ζ are expressed in spinal deformities caused by mechanical loading, and their expression depends on the type and severity of the applied strain.

1. Introduction

Spinal deformities are often associated with structural defects of both intervertebral discs (IVD) and vertebrae end plates (EP) that alter the tissue architecture, metabolic activity and matrix integrity. The biomechanical properties of IVD enhance the load bearing capacity and mobility of the spine. EP, which acts as slow-growing epiphyses, is responsible for the growth of the anterior column of vertebrae, which is important for its longitudinal growth [1]. Additionally, EP absorbs considerable hydrostatic pressure from spine loads [2] and interferes with the IVD nutrition pathways [3], being critical for maintaining disc function and integrity [4].

Numerous potential causes of spinal deformities have been investigated, including genetic inheritance [5,6], disorders of the nervous system, connective tissue and muscle abnormalities [5], and hormonal imbalance [5,7]. The implication of altered biomechanical environment as a major factor associated with spinal deformities onset and progression, has been supported by several in vivo studies [8–10]. It has been shown that repetitive loading directly provokes disc deformation, resulting in a decreased pH and proteoglycans content, an increased osmolarity, and an altered cell metabolism [11]. Overloading also induces EP damage, which leads to disc degeneration [11]. In the vertebrae EP, cell proliferation and matrix metabolism are regulated by several growth factors [12]. Similarly, in the IVD, tissue homeostasis is maintained

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by a balance between catabolic and anabolic regulator polypeptides [13].

A growth and differentiation factor that has been detected within the epiphyseal plate of mouse [14] and within human disc tissue [15] is pleiotrophin (PTN). PTN, also named osteoblast-specific protein 1, is a secreted growth factor with well-established roles in the development and maturation of the nervous system, as well as in tumor growth and angiogenesis [16]. Its expression in the fetal and infant cartilage of animals, as well as in the developing bone, suggests a potential role in cartilage and bone formation or remodeling [17]. PTN exerts its biological action through its receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ). RPTPβ/ζ is expressed in differentiated osteoblasts that guide bone formation in mice [18], but its expression either in the EP or in the IVD has never been examined.

The purpose of the present study was to examine the expression of PTN and RPTPβ/ζ in the convex and the concave side of the whole functional unit of IVD and vertebrae EP, after the application of axial compression and gradually increased asymmetrical loading, using an Ilizarov mini external fixator in a rat model.

2. Methods

2.1. Experimental design and surgical procedures

The experimental protocol was approved by the General Directorate of Veterinary Services (license number: K/2314/16-06-2011), according to Greek legislation (Presidential Degree No. 160/1991) with which Greece has conformed to the Directive 86/609/EEC regarding the protection of vertebrate animals used for experimental or other scientific purposes. In total, sixty four Sprague-Dawley rats, aged eight weeks old and weighing 206 ± 15 g (mean ± s.d.) were obtained from the registered breeding unit (EL 25 BIO 011) of the Hellenic Pasteur Institute (Athens, Greece). The animals were housed two to a cage (transparent polycarbonate 45 × 30 × 20 cm, IFFA), under standard laboratory conditions, with temperatures ranging between 19 °C and 22 °C, relative humidity between 55% and 65%, 15 air changes/h, and a light/dark cycle at 06:00/18:00 h. The rats were anesthetized by intramuscular injection of ketamine hydrochloride 50 mg/kg and dexmedetomidine 0.5 mg/kg. Chemoprophylaxis (cefuroxime 30 mg/kg i.m.) and analgesia (carprofen 5 mg/kg s.c.) were administered preoperatively, as well as on the first two postoperative days. The caudal vertebrae for the placement of the mini-Ilizarov apparatus were selected, following which, using aseptic procedures, they were drilled through and the pins inserted under fluoroscopic control. The connective rods were adjusted to align the rings of the parallel, imposing a compressive stress, or at an angle. The external fixator was installed, for 5 weeks between the 9th and 10th vertebrae [19,20]. Following surgery, anesthesia was reversed by the administration of atipamezole 0.2 mg/kg i.m. and recovery of the animals was smooth and uneventful. The rats were monitored postoperatively for signs of regional inflammation, anorexia, normal activity and discomfort toward the apparatus. The animals behaved in a normal way after the first 5 postoperative days. In a small percentage of animals, mild regional swelling of the tail skin was observed in the first postoperative days and successfully treated with the topical application of Betadine solution for 4–8 days. No signs of pus or necrosis were noted. According to the degree of the deformity, the animals were categorized in four groups

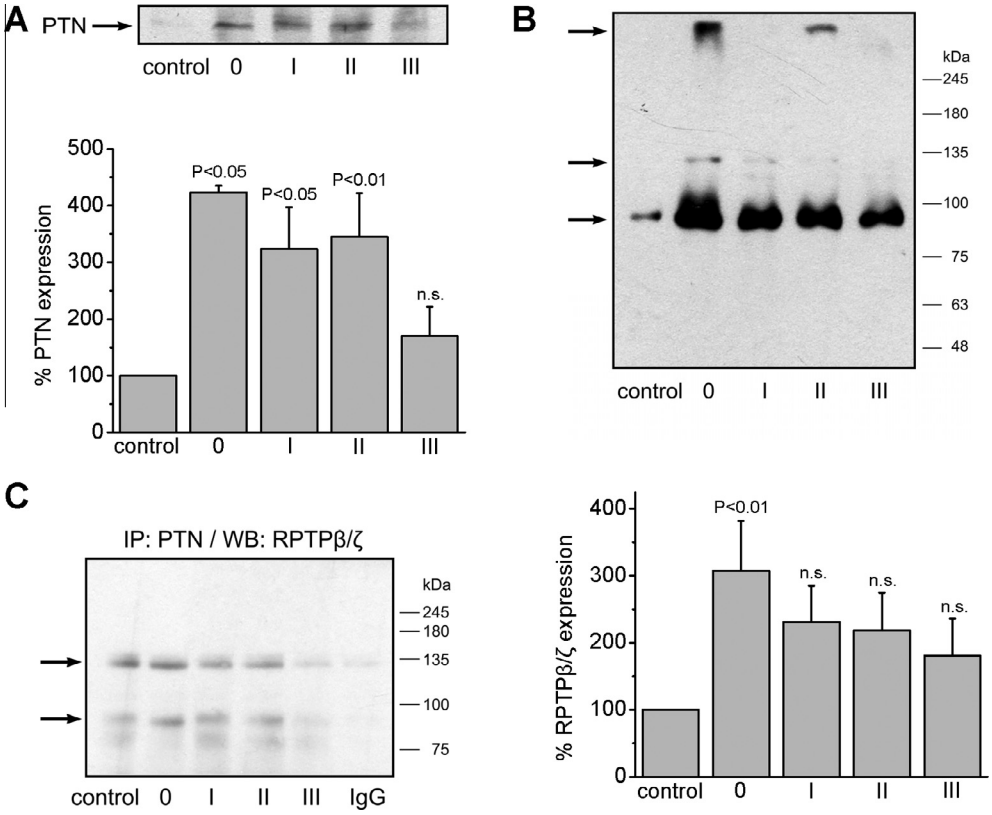

Fig. 1. Mechanical loading increased PTN and RPTPβ/ζ expression in the whole of IVD and EP areas. Representative picture of Western blot analysis for PTN (A) and RPTPβ/ζ (arrows mark different RPTPβ/ζ isoforms, see text) (B) from the EP and IVD of the rat tails subjected to 0°, 10°, 30° or 50° axial compressions (groups 0, I, II and III respectively). In both cases, the immunoreactive bands were quantified by densitometric analysis and the results are expressed as mean ± s.e.m. of the percent PTN or RPTPβ/ζ levels in each group compared with the control group (set as 100%). Statistical significance compared with the control group is shown; n.s., not significant. (C) Representative picture of Western blot analysis for RPTPβ/ζ of immunoprecipitated for PTN samples from the EP and IVD of the rat tails.