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Insulin-deficient diabetes-induced bone microarchitecture alterations are associated with a decrease in the osteogenic potential of bone marrow progenitor cells: Preventive effects of metformin

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ABSTRACT

Aims: Diabetes mellitus is associated with metabolic bone disease and increased low-impact fractures. The insulin-sensitizer metformin possesses in vitro, in vivo and ex vivo osteogenic effects, although this has not been adequately studied in the context of diabetes. We evaluated the effect of insulin-deficient diabetes and/or metformin on bone micro-architecture, on osteogenic potential of bone marrow progenitor cells (BMPC) and possible mechanisms involved.

Methods: Partially insulin-deficient diabetes was induced in rats by nicotinamide/strepto-zotocin-injection, with or without oral metformin treatment. Femoral metaphysis micro-architecture, ex vivo osteogenic potential of BMPC, and BMPC expression of Runx-2, PPARγ and receptor for advanced glycation endproducts (RAGE) were investigated.

Results: Histomorphometric analysis of diabetic femoral metaphysis demonstrated a slight decrease in trabecular area and a significant reduction in osteocyte density, growth plate height and TRAP (tartrate-resistant acid phosphatase) activity in the primary spongiosa. BMPC obtained from diabetic animals showed a reduction in Runx-2/PPARγ ratio and in their osteogenic potential, and an increase in RAGE expression. Metformin treatment prevented the diabetes-induced alterations in bone micro-architecture and BMPC osteogenic potential.

Conclusion: Partially insulin-deficient diabetes induces deleterious effects on long-bone micro-architecture that are associated with a decrease in BMPC osteogenic potential, which could be mediated by a decrease in their Runx-2/PPARγ ratio and up-regulation of RAGE. These diabetes-induced alterations can be totally or partially prevented by oral administration of metformin.

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1. Introduction

In recent years there has been a growing interest in the association between diabetes and alterations in bone metabolism, which has given rise to substantial research on the effects of diabetes and of anti-diabetic drug intake on bone health [1]. Type 1 diabetes has been clearly associated with low bone mass and a 6-fold increase in low-impact fractures. On the other hand, the effects of type 2 diabetes on bone are less clear-cut: whereas bone mineral density has been reported to be either unchanged or modestly increased, incidence of low-stress fractures is almost doubled, pointing to a decrease in the bone quality of these patients [2–4]. Additionally, in both types of diabetes there is delayed fracture healing [5]. Diabetes-associated alterations in bone quality and repair have been related to poor glycemic control, to an increase in reactive oxygen species (ROS) production, and to bone matrix accumulation of advanced glycation endproducts (AGEs) [6–8]. In spite of all these reports, the underlying mechanisms of diabetes-induced bone alterations are incompletely known.

AGEs accumulation in the extracellular matrix alters bone mechanical properties through the excessive formation of nonenzymatic crosslinks between collagen chains, thus generating irreversible intra- and inter-molecular covalent bonds [9]. Additionally, collagen AGEs accumulation also induces alterations in bone cell homeostasis, mainly due to the specific recognition of AGEs by receptors such as RAGE that in osteoblasts activate intracellular signaling pathways leading to a decrease in free IGF-1 levels, and to a reduction in osteoblastic proliferation, differentiation, mineralization and survival [10–15]. Interestingly, AGEs recognition by RAGE up-regulates the expression of this receptor, thus providing a positive feedback loop that can increase the osteoblastic response to extracellular AGEs accumulation [16–18].

Osteoblasts and adipocytes derive through divergent and mutually inhibitory processes, from a common pluripotent precursor: the bone marrow progenitor cell (BMPC) of mesenchymal origin. BMPC osteoblastic differentiation is induced by the transcription factor Runx2 [19], while adipocytic differentiation is controlled by peroxisome proliferator-activated receptor-γ (PPARγ) [20]. Rodent models of type 1 diabetes show an increase in bone marrow adiposity and PPARγ activity, and a decrease in osteoblastic density with low bone mass, suggesting that their bone phenotype could be the consequence of an increase in the expression of PPARγ by BMPC [21]. In type 2 diabetes, BMPC phenotypic commitment to the osteoblastic/adipocytic lineage has not been established to date.

The possible effects on bone metabolism of insulin-sensitizing agents have also been studied. Thiazolidinediones induce BMPC adipogenesis and thus bone marrow adiposity, by binding to PPARγ2 receptors and enhancing expression of the adipogenic transcription factor aP2 [20,22]. Treatment of mice with thiazolidinediones decreases bone mineral content, bone formation, and trabecular bone volume, by decreasing Runx2 expression [22,23]. On the other hand, we have previously demonstrated that the biguanide metformin, widely used for treatment of type 2 diabetes, has osteogenic effects. In vitro, metformin can directly stimulate the proliferation, differentiation and mineralization of osteoblasts [24]. Metformin can also prevent the deleterious effects of AGEs on osteoblasts in culture, in part by blocking the AGEs-induced up-regulation of RAGE [17]. Oral administration of metformin to non-diabetic animals stimulates the osteogenic potential of BMPC and improves bone healing, by increasing the expression of Runx2 [25]. Due to its antioxidant and insulin-sparing effects, as well as its ability to improve cardiovascular outcomes, metformin is presently under evaluation as an adjunct therapy in patients with type 1 diabetes mellitus [26].

Based on these considerations, we hypothesized that diabetes-induced deleterious effects on bone metabolism, particularly alterations in long bone micro-architecture, could be partly due to a decrease in the osteogenic potential of BMPC; and that these alterations could be prevented by metformin treatment. To prove our hypothesis, we evaluated the effect of partially insulin-deficient diabetes and/or oral metformin administration in rats, on femoral metaphysis micro-architecture and BMPC osteogenic potential. We also studied possible mechanisms of action, such as expression of RAGE, Runx-2/PPARγ ratio, and pro-inflammatory cytokine levels.

2. Materials and methods

2.1.1. *Animal treatments*

Two-month-old male Sprague-Dawley rats (190–210 g) were used. Animals were maintained in a temperature-controlled room at 23 °C, with a fixed 12 h light: 12 h darkness cycle, and fed standard rat laboratory chow and water ad libitum. All experiments on animals were done in conformity with the Guidelines on Handling and Training of Laboratory Animals published by the Universities Federation for Animals Welfare [27]. Approval for animal studies was obtained from the institutional accreditation committee (INIBIOLP's Animal Welfare Assurance No A5647-01). In half the animals, partially insulin-deficient diabetes mellitus was induced by i.p. injection of nicotinamide (NA) (50 mg/kg) in physiological saline followed by i.p. streptozotocin (STZ) (60 mg/kg) freshly dissolved in citrate buffer (0.05 M, pH 4.5) [28]. One week later, blood glucose was assayed to verify diabetes in all NA-STZ-treated animals. Animals were then divided into four groups of 10 animals per group: control (non-treated non-diabetic) (C) and diabetic rats (D) received water ad libitum; metformin-treated non-diabetic (M) and diabetic rats (D-M) received 100 mg/kg/day of metformin (Química Montpellier, Buenos Aires, Argentina) in drinking water for 2 weeks. After all treatments non-fasting blood samples were taken, and serum was stored at −20 °C until biochemical evaluation. Serum glucose and triglycerides were measured by commercial kits (Wiener Laboratories, Argentina), insulin by a rat-specific ELISA kit from ALPCO and fructosamine by a colorimetric BioSystems kit. Serum pro-inflammatory status was assessed by evaluation of plasmatic TNFα by an ELISA kit (BD OptEIA™ mouse TNF (Mono/Mono) as we have previously described [29].