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Regeneration and healing of bone and cartilage in type-1 and type-2 diabetes: the effects of insulin

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Abstract: Increased fragility of long bones and delay in fracture healing in adults as well as skeletal development disorders in children are thought to be associated with endochondral ossification problems. The extracellular matrix (ECM) of cartilage is absorbed during endochondral ossification. During the recovery process from damage, chondrocytes undergo hypertrophy and increase their cell volume; on the other hand, it is also necessary to digest the ECM elements around the cells. Recent research on the proteolytic enzymes in ECM assumed to be responsible for the digestion is connected to proteinases digesting type II collagen and aggrecan. ADAMTS1, 4, and 5 are known as major aggrecanases within the matrix metalloproteinase (MMP) enzymes in human cartilage. These are regarded as main determiners among MMPs of bone growth, healing, and remodeling. In this mini review article, the remodeling and restoration of osseous tissue in type-1 and -2 diabetes mellitus (DM) and the effect of insulin on these processes are discussed briefly in the light of the current literature.

Key words: Bone, cartilage, healing, fracture, growth, aggrecan, aggrecanase, ADAMTS, metalloproteinase

1. Introduction

Glucose is an essential nutrient in terms of metabolism and structural needs in the hyaline cartilage and bone growth centers. Glucose is a primer substrate for the production of ATP for the chondrocytes in an anaerobic environment; moreover, it is also the source of glucosamine sulfate for the development, protection, repair, and remodeling of cartilage (Mangiavini et al., 2014). Decrease in linear growth in the skeletal system and abnormalities in the insulin-like growth factor-1 (IGF-1) axis were noted in children with uncontrolled type-1 diabetes mellitus (type-1 DM) (Chiarelli et al., 2004; Bizzarri et al., 2014). In these patients, some health problems occur during growth and development during the period of entry into adolescence. On the other hand, patients with type-2 DM are known to have problems with wound healing, bone resistance, healing of fractured bones, and poor recovery from tooth implant surgery.

The fact that insulin stimulates matrix synthesis suggests modulation of chondrocyte metabolism via multiple biosynthetic/receptor pathways (Glade et al., 1994). Insulin is recognized by all target organs via the insulin receptor (IR) and IGFR-1. IGF-1, a major stimulator of matrix synthesis in cartilage, is likely to play a role in replacing lost or damaged cartilage matrix during bone remodeling (Martin et al., 2000). Bone formation and remodeling is a dynamic process that requires energy expenditure. Therefore, one of the major targets for insulin signaling is bone cells. A molecule obligatory for bone tissues, Runx2, was found to be induced in 3T3-L1 cells (Adhami et al., 2011). Runx2-null cells were at an elevated state of energy metabolism and addition of insulin resulted in a marked suppression of genes required for insulin signaling. The suppression was observed at all stages of the insulin pathway (receptors, transducers, nuclear effectors, and target genes) (Adhami et al., 2011).

2. The effects of type-1 and -2 DM on physiological and pathological processes of connective tissue

2.1. Skeletal growth

One of the most important results in children with untreated type-1 DM is somatic growth retardation (Maor and Karnieli, 1999; Giannini et al., 2014), because insulin has a central role in the main regulation of the GH/IGF axis. Successful insulin regimens applied to this type of patient have the advantage of more physiological circulating insulin concentrations, resulting in improving GH/IGF alterations. Overweight children are taller than their normal weighted peers (Stovitz et al., 2010; Olza et

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al., 2014). The skeletal growth in these children occurs at an earlier stage and they reach adolescence at earlier ages. Although clinical and experimental evidence support the growth-promoting properties of insulin (Fennoy et al., 2013), it is not yet known whether insulin resistance or hyperinsulinemia is responsible for rapid structural growth and skeletal maturation in children. It is found in mice fed a high-fat diet that insulin resistance and accelerated skeleton growth are related. During in vitro experiments, it is shown that insulin itself directly modulates skeleton growth in the growth plate by activating insulin receptors (Phornphutkul et al., 2006). Regarding type-2 DM, insulin resistance has been suggested to be a core defect in the pathophysiology of obesity-related comorbidities in children (Shaibi et al., 2006). However, it has been shown that exercise training can improve insulin resistance and reduce the risk of type-2 DM in adults. It is not known whether exercise has an effect on insulin resistance in children (Shaibi et al., 2006).

2.2. Difficulties in dental operations

Maxillary sinus graft application is claimed to be contradictory when used for tooth implants in patients with uncontrolled DM (Nevins et al., 1998). It is found in animal studies that type-1 DM decreases bone formation and results in osteopenia and delayed fracture healing (Lu et al., 2003). In type-1 DM, bone formation is delayed after fracture of the tibia and femur, and this effect was reversed upon insulin replacement (Beam et al., 2002). The new formation of bone areas, the number of osteoblasts, and the amount of collagen were decreased in diabetic rats operated on for maxillary sinus graft (Hou et al., 2012). Serum osteocalcin levels were also found to be decreased. Insulin application leads to an increase in osteogenesis. It demonstrates that decreased bone formation in type-1 DM animal models might be prevented by insulin therapy.

2.3. Bone healing

There are several components of bone fracture healing: hematoma, migration and proliferation of progenitor cells to the affected area, and differentiation into osteoblasts and chondrocytes. The second classes of cells, namely chondrocytes, manufacture the cartilage tissue, which in turn produces first cartilaginous and then calcified tissue. In the environment, osteoblasts start to form endochondrial bone. The reason for osteopenia in type-1 DM is the decreased bone mineral density and bone formation (Gerstenfeld et al., 2005). Patients with type-1 and -2 DM also display prolonged wound healing compared to healthy counterparts (Chaudhary et al., 2008). Both type-1 and -2 DM are accepted as among the main metabolic disorders, increasing fracture predisposition and impairing fracture healing as a consequence of hyperglycemia and increased advanced glycation end products formation, ROS generation, and inflammation (Jiao et al., 2015). It was reported that the adverse effects of type-2 DM on bone healing in rats are mainly attributed to the improving osteoblast functioning and bone formation processes (Hamann et al., 2011). Moreover, suppressed osteoblastogenesis as a cause and mechanism for low bone mass and impaired bone healing is also a challenge for type 2 DM. Contrary to the common belief, cartilage metabolism is the main crucial determiner of fracture healing rather than bone metabolism in terms of the beginner events in the healing process of bone fractures. The subject is rather original, and needs to be studied in type-1 and -2 DM since the process is delayed, and differentiation and proliferation decline in type-1 and -2 DM (Gandhi et al., 2005).

Contemporary findings in the regeneration and restoration of bone remodeling emphasize matrix metalloproteinases (MMPs) because mice lacking these MMPs have huge defects in bone machinery (Aiken et al., 2010; Uysal et al., 2013). The way the MMPs works is a simple one: they cut their own substrates at certain points. They are also responsible for communications between certain bone cells.

3. A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs (ADAMTS)

ADAMTS enzymes (Akyol et al., 2015b) are divided into three classes according to their capacity to break the aggrecan molecule: Aggrecanase-1: ADAMTS1 and 4; Aggrecanase-2: ADAMTS5; Aggrecanase-3: ADAMTS8, 9, and 15. Of those, ADAMTS4 and 5 show the most powerful aggrecanase activities (Tortorella et al., 1999; Rogerson et al., 2008). It has been proven that ADAMTS proteins play crucial roles in growth development, the metabolism of the cartilage extracellular matrix (ECM), and progression of joint diseases (Arner et al., 2002). ADAMTS4 and 5 are known as major aggrecanases within the MMP in human cartilage. The activation process of this naturally found zymogen enzyme is not entirely known, and needs to be clarified. Although the roles of ADAMTS in chondrocyte growth, differentiation, and maturation on the epiphyseal plate are known to an extent, their roles in endochondral ossification are not fully proven yet (Demircan et al., 2005; Hatipoglu et al., 2009; Yaykasli et al., 2009).

3.1. Direct insulin effects on ADAMTS

Researchers have shown the mRNA expression of ADAMTS9 during the chondrogenesis of insulin-induced ATDC5 cells with RT-PCR. As a result, ADAMTS9 is expressed in these chondrocytes, and together with the proliferation of chondrocytes and matrix degradation, they help to contribute to hypertrophy of chondrocytes and the endochondral ossification process (Kumagishi et al., 2009). After in vitro induction of chondrogenesis by insulin in ATDC5 cells, the ADAMTS9 expression

is bimodally increased: the first peak occurs between days 8 and 14 and the second between days 28 and 35. However, the type X collagen, a marker of hypertrophic chondrocytes expression, has shown its peak on day 22. Type-1 and -2 DM deteriorates cartilage formation and resorption as well as overall fracture healing extensively. Yet, the molecular mechanism remains obscure. The effects of insulin for these processes require both in vivo and in vitro experimental setup studies. The relationship among ECM formation, cartilage production, and ADAMTS enzyme levels/activities as well as tissue inhibitory proteins in bone formation needs to be enlightened in both protein and mRNA levels (Demircan et al., 2013). The main reason for increased fragility of long bones in patients with unregulated type-1 and -2 DM may be insulin's absence/ineffectiveness. The reducing/activity inhibiting effect of insulin on ADAMTS proteins detected in our recent unpublished study ensures that the cartilage and bone tissues under insulin depression prefer going in an anabolic direction. In insulin absence, however, it can be anticipated that an increase in ADAMTS activity may accelerate the destruction of ECM in cartilage and bone tissues leading consequently to a reduction in the resistance to bone fractures. According to one of our previous studies, ADAMTS6 (one of the orphan ADAMTS) mRNA expression was found to be decreased as early as one day after insulin application, and continued up to day 11 (Uğurcu et al., 2014). It was shown that animals with type-1 DM created smaller fracture callus compared to controls (Follak et al., 2004). The healing time interval and recovery time are prolonged dramatically in type-1 DM (White et al., 2003; Gandhi et al., 2005). Molecular studies revealed that DNA amount inside healing fractures in type-1 DM is reduced up to 40% (Brown et al., 2014), and collagen content in newly growing callus was decreased in animals with type-1 DM compared to normoglycemic ones (Brown et al., 2014).

When insulin was induced into Swarm rat chondrocyte cells, the insulin receptors increased, which is not interpreted as an increase in the de novo receptor synthesis or a translocation of other intracellular receptors located in the cell compartments close to the cell surface, but most likely as a decrease in the rate of receptor degradation (Otsu et al., 1988). On the other hand, experiments with human recombinant insulin by ruling out all other possibilities have shown that increased proteoglycan synthesis is entirely caused by insulin itself (Otsu et al., 1988).

3.2. General behavior of MMPs on ECM degradation

A loss of chondrocytes is considered physiologically significant since the loss itself triggers the production of several signals, which stimulates cartilage resorption. In hypertrophic chondrocytes, MMP13, which digests fibrillary collagen and aggrecan, is normally expressed (Cawston et al., 2006). MMP13 knockout mice show a phenotype in which chondrocytes undergo regular hypertrophy leading to a delay in ossification invasion (Inada et al., 2004). ADAMTS1, 4, and 5 knockout mice are able to grow up normal, and do not show any defect in their cartilaginous tissues (Little et al., 2005; Stanton et al., 2005). Because these are the most important aggrecanases found in the cartilage, none of them take part alone in the removal of cartilage aggrecan in a significant role. ADAMTS1 gene is responsive to inflammation that is not expressed in normal tissues, but it is induced by lipopolysaccharide stimulation (Kuno et al., 1997). ADAMTS1 and 4 play a crucial role in versican proteolysis (Sandy et al., 2001). Procollagen and proteoglycan substrates were recognized by ADAMTS proteases with a great specificity (Sandy et al., 2001). Expanding chondrocytes are facilitated by some other proteinases such as cathepsins and calpains, which remove the cartilage ECM surroundings (Cawston et al., 2006).

The most important extracellular cartilage compound is aggrecan, which is a proteoglycan, providing strength against pressure force, dynamic weight-bearing function, and an osmotic feature (Akyol et al., 2014a). As an incredibly complex macromolecule, aggrecan is specific to the cartilage and intervertebral disc, and is extremely hydrophilic, consisting of glycosaminoglycan chains containing about 100 chondroitin sulfate units and 30 keratin sulfate chains (Hardingham et al., 1992). Normal and osteoarthritic cartilage normally express ADAMTS4 (Yamanishi et al., 2002). If inhibited by some factors in vitro, the collapse of aggrecan is prevented (Malfait et al., 2002). Knockout mice with ADAMTS4 and 5 dual deletions can phenotypically differ from the wild-type.

Numerous biological factors were determined, affecting MMP activities including cytokines, hormones, growth factors, and many others (Demircan et al., 2014). MMPs have been inhibited by two types of proteinase inhibitors: Tissue inhibitors of metalloproteinases (TIMPs) and secreted inhibitors of metalloproteases (IMPs) (Mandal et al., 2003). TIMPs are natural inhibitors important for various physiological process controls. These processes include cell invasion, angiogenesis, digestion of articular cartilage, trophoblast implantation, inner folds formation of mammary gland, and wound healing (Twining et al., 1994; Demircan et al., 2014). TIMP-2 drives tissue proteinases to stop, and thus the structure remains intact.

4. Signaling pathway of insulin in chondrosarcoma cells

Chondrosarcoma cells are one of the most convenient cell types to study cartilage metabolism; however, the signaling pathway of insulin in these cells has rarely been studied to compare aggrecan degradation with others. When insulin is supplemented to the ATDC5 cell media, it

causes cell condensation and consequently chondrogenesis (Shukunami et al., 1997). PI3K/PKB (Akt) is known to be involved in insulin/IGF-1-induced chondrogenesis (Hidaka et al., 2001). The activation of PI3K and PI3 production results from the phosphorylation of tyrosine residues by insulin stimulation via insulin receptor substrate-1 (IRS-1) (Sparks et al., 2013). Insulin/IGF-1 pathway is assigned to lead to chondrogenic differentiation in ATDC5 cells (Hidaka et al., 2001).

Molecular, histological, and immunohistological studies revealed that diabetes itself caused increased osteoclastogenesis and mainly loss of cartilage and increased elevated mRNA levels of various pro-resorptive elements of cartilage loss. All of these parameters totally or individually were reversed by treatment with insulin (Kayal et al., 2009). Specifically, the impact of type-1 DM and insulin application on cartilage was examined in the above-mentioned study. Ten days after fracture, the normoglycemic and insulin treated diabetic animals had 1.6-fold more cartilage than the diabetic ones. The increased osteoclast numbers were also reversed by insulin treatment. In type-1 DM, increased TNF-alpha and receptor activator of nuclear factor kappa-B ligand (RANKL) expression and rapid removal of cartilage were observed. Upon slow release insulin treatment, these diabetes-induced TNF-α and RANKL expressions were reversed.

Insulin stimulates proteoglycan synthesis in the chondrocytes by acting through the insulin receptors (Foley et al., 1982). This effect occurs at physiological concentrations in the Swarm rat chondrosarcoma chondrocytes by insulin receptors themselves rather than pharmacological concentrations. One of the reasons is that a considerable amount of anti-insulin receptors were found to inhibit the biological response to the insulin-like growth factors. The other reason is that insulin at physiological concentrations stimulates sulfate incorporation into macromolecules in chondrocyte cultures derived from the Swarm rat chondrosarcoma. Sulfate is needed for the synthesis of some amino acids to be able to participate in the synthesis of various kinds of ECM structures, which have complex proteins (Bogdani et al., 2014).

5. Conclusion

Many studies are focused on the effects of several molecules to elucidate the matrix biology of chondrocytes and pathophysiology of experimental osteoarthritis (Akyol et al., 2013, 2014b). A list of the beneficial effects of insulin (Akyol et al., 2015a; Altuntaş et al., 2015) discussed in detail via whether its regulating effect on hyperglycemia or its direct effect on cells may be as follows: accelerating fracture healing, improving resistance to fractures, providing growth and development of skeletal structures in children, and ensuring success with pre-operational processes in dental implant surgery. Insulin does not necessarily participate in all stages of ADAMTS metabolism. We previously reported that insulin does not participate in regulation of ADAMTS13 levels and/or activities in OUMS-27 human chondrosarcoma cells (Firat et al., 2014). Future research on ADAMTS and TIMP in terms of gene, mRNA, and protein phases in animals with experimentally induced type-1 DM would possibly help show the full-scale machinery of the ECM in the cartilage.

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