Metformin in Diabetic and Non-Diabetic Bone Impairment

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Metformin is a widely-used anti-diabetic drug in patients with type 2 diabetic mellitus (T2DM) due to its safety and efficacy in clinical. The classic effect of metformin on lowering blood glucose levels is to inhibit liver gluconeogenesis that reduces glucose production as well as increases peripheral glucose utilization. However, the factors such as hyperglycemia, insulin deficiency, reduced serum levels of insulin-like growth factor-1 (IGF-1) and osteocalcin, accumulation of advanced glycation end products (AGEs), especially in collagen, microangiopathy, and inflammation reduced bone quality in diabetic patients. However, hyperglycemia, insulin deficiency, reduced levels of insulin-like growth factor-1 (IGF-1) and osteocalcin in serum, accumulation of advanced glycation end products (AGEs) in collagen, microangiopathy, and inflammation, reduce bone quality in diabetic patients. Furthermore, the imbalance of AGE/RAGE results in bone fragility via attenuating osteogenesis. Thus, adequate glycemic control by medical intervention is necessary to prevent bone tissue alterations in diabetic patients. Metformin mainly activates adenosine 5′ -monophosphate-activated protein kinase (AMPK), and inhibits mitochondrial respiratory chain complex I in bone metabolism. In addition, metformin increases the expression of transcription factor runt-related transcription factor2 (RUNX2) and Sirtuin protein to regulate related gene expression in bone formation. Until now, there are a lot of preclinical or clinical findings on the application of metformin to promote bone repair. Taken together, metformin is considered as a potential medication for adjuvant therapy in bone metabolic disorders further to its antidiabetic effect. Taken together, as a conventional hypoglycemia drug with multifaceted effects, metformin has been considered a potential adjuvant drug for the treatment of bone metabolic disorders.

metformin AMPK RUNX2 AGEs osteogenesis

1. Effects of Metformin on Cells In Vitro

1.1. Effects on Stem Cells

It is well known that the osteogenic potential of mesenchymal stem cells (MSCs) is seriously affected by persistent inflammation of periodontitis. Metformin carbon dots (MCDs) including citric acid and metformin hydrochloride effectively improved the activity of alkaline phosphatase (ALP), the formation of calcium deposition nodules, the expression of genes and osteogenic proteins in mesenchymal stem cells of the rat bone marrow (rBMSCs), thereafter effectively helped to regenerate lost alveolar bone in periodontitis rats [1]. A recent study showed that polycaprolactone/chitosan nanofibrous membranes containing metformin would be favored for bone regeneration as guided bone regeneration membranes because it was more suitable for cell proliferation, adhesion, and

osteogenic differentiation of rBMSCs [2]. A study from canine BMSCs showed that metformin was a better osteogenic inducer for osteogenic differentiation in vitro [3]. Additionally, Metformin significantly induced osteogenic differentiation of human BMSCs while it was attenuated via inhibiting phosphorylation of glycogen synthase kinase 3b (GSK3b), which suggested that metformin had a potential effect on stimulating differentiation of human MSCs toward osteoblast [4].

1.2. Acts on Other Bone Cells

Metformin inhibited BMP6-induced Smad1/5 phosphorylation in osteoblast MC3T3-E1 cells, along with Smad6 up-regulation, and this effect was mitigated by the knockdown of Smad6 [5]. Thus, metformin may be a potential therapeutic drug for trauma-induced heterotopic ossification. A novel elastomeric biodegradable bone regenerative films developed from metformin and polyurethane (PU) exhibited that metformin present in PU formulation promoted adhesion, proliferation, and calcium deposition of MC3T3-E1 cell line [6]. In addition, metformin enhanced osteoblastic cell mobility in wound healing and migration assay and upregulated mark protein expression in osteoblastic differentiation in U2OS and MG63 cells while suppressing the differentiation of osteoclast in Raw 264.7 cells, and protected against ischemic necrosis in the rat femoral head epiphysis by preserving osteocyte function [7]. Metformin-treated preosteoblasts increased the expression of OPN which reduced the subsequent adherence of myeloma cells when they were silent. Proliferation markers were reduced in cocultured myeloma cells with preosteoblasts treated with metformin. Mice with 5TGM1 myeloma cells pre-treated with metformin had increased tumor loads, associated with increased osteolytic bone damage and high expression of the OPN in the bone marrow [8]. In an in vitro study of nondiabetic rats with a cranial defect model, metformin promoted the differentiation of rat adipose tissue (rASCs) into bone-forming cells. which osteogenic effect of metformin was also demonstrated with the rich calcium and phosphorous deposits on the newly formed mineralized extracellular matrix. [9].

2. Research on Bone Defect Animal Models

2.1. Promote Alveolar Bone Repair

In a critical-size alveolar bone defects model of rats, the Gelatin/nano-hydroxyapatite/metformin scaffold showed superior bone regeneration and promoted the synthesis of osteogenic proteins such as osteocalcin (OCN), osteonectin, and collagen type I, which may be applied as a potential bone substitute to regenerate alveolar bone due to its good biocompatibility, interconnected pores allowing vascularization, relatively fast degradation, and higher bioactivity properties [10]. In a periodontitis rat model, local administration of chitosan-metformin based intra pocket dental film led to the reduction of alveolar bone destruction and displayed good antibacterial activity [11]. Additionally, the metformin-loaded b-tricalcium phosphate/Chitosan/mesoporous silica scaffolds implanted in the region of alveolar bone malformations in rats suffering from periodontitis promoted alveolar bone regeneration [12]. In another bone tissue engineering study, the group with metformin plus osteogenic had three- to four-fold increases over those of the osteogenic alone group in osteogenic gene expressions, ALP activity, and mineral synthesis, which demonstrated that human periodontal ligament stem cell (hPDLSCs) was a potent cell source for

bone engineering and the calcium phosphate cement (CPC)-metformin scaffold with hPDLSCs was a highly promising construct to promote bone repair and regeneration effectively in craniofacial, dental, and orthopedic applications [13]. Thus, metformin might be an additional osteoinductive factor in osteogenesis.

2.2. Enhance Tendon-Bone Interface Healing

The healing of the tendon-bone interface (TBI) is a clinical dilemma that is closely related to the forming and remodeling of new bones at the repair site. A canine model study showed that the Achilles tendon-calcaneus (ATC) interfaces treated with metformin were repaired with a significantly higher fracture load and stiffness than the metformin-free test site. The micro-computed tomography (CT) analysis showed that the metformin-treated samples exhibited significantly higher bone volume/total volume and trabecular thickness than those of the metformin-free controls. These results were confirmed by hematoxylin and eosin (HE) staining as well. Immunohistochemical (IHC) staining showed that there were considerably more cells with OCN in newly formed bones with metformin-treated than those in the metformin-free control site at week 4. Furthermore, Masson's trichrome staining showed that significantly more oriented collagen fibers anchored in the newly formed bone of the metformin-treated site than in the metformin-free control site [4]. Consequently, the local administration of metformin provided bone microarchitecture improvement at the calcaneus and an increase in the tensile properties of the repaired ATC interfaces in canines. These important findings demonstrated that the local administration of metformin may be an effective strategy for TBI healing in clinic.

2.3. Single Use of Metformin in Bone Repair

In a rat model of TiAl6Va4 implants on tibial bone, the ratio of peri implant bone tissue filling was higher in the metformin group than those in the control group, which suggested that systemic administration of metformin might increase titanium implant osseointegration in non-diabetic rats [14]. A poly lactic acid and polycaprolactone scaffold with the delivery of metformin-loaded gelatin nanocarriers enhanced the expression of the markers of osteogenic and angiogenic considerably and ameliorate bone in angiogenesis, growth, and defect reconstruction in a rat model of calvarial bone defects [15]. Additionally, metformin can accelerate bone healing and mature tissue formation at a fracture site in a cranial defect rats' model [16]. In collagen-induced arthritis (CIA) model rats, metformin significantly inhibited systemic inflammation and synovitis, the changes of trabecular bone and degradation of the cartilage layer matrix, and osteoclast formation in the knee joint, and the apoptosis of chondrocytes [16]. In a chronic kidney disease-mineral and bone disorder (CKD-MBD) rat model, metformin protected against the development toward severe CKD to prevent vascular calcification development and high bone turnover disease progression, but there was no evidence of the reduction of aorta or small vessel calcification [17].

Moreover, metformin effectively increased the levels of serum ALP in the ketogenic diet (KD) mice while reducing the levels of serum TRAP in the OVX mice, but the OCN expression up-regulated and the TRAP expression down-regulated in both OVX and KD mice [18]. This research revealed that metformin can effectively alleviate KD-induced cancellous bone loss and maintain the biomechanical properties of long bones, which suggested that metformin

was a potential drug for the treatment of KD-induced osteoporosis in teenage [18]. In ultra-high-molecular-weight polyethylene particle-induced osteolysis mouse models, metformin reduced dickkopf-related protein 1 (DKK1), and sclerostin that is the negative regulator of bone formation, and increased OPG secretion and the ratio of OPG/RANKL to exert the property of bone protect [19]. These findings suggested that metformin-induced differentiation and mineralization of osteoblasts, while it inhibited osteoclastogenesis through the secretion of mature osteocytes [19]. A systematic review was conducted in accordance with the 2020 PRISMA guidelines to evaluate the evidence supporting the bone-protective effects of metformin on male animal models with T2DM.

This research shows that metformin enhanced bone density and reduced the effects of T2DM on fat formation in animal models, however, further research is needed to determine the optimal dose of metformin needed to show these therapeutic effects [20].

2.4. Combinational Use of Metformin in Bone Repair

A novel poly L-lactic acid/nanoscale hydroxyapatite/metformin nanocomposite scaffold had the dual function of tumor repression and bone repair, which provides a promising new therapy for tumor-induced bone defects [21]. The metformin-incorporated nano-gelatin/hydroxyapatite fibers upregulated osteogenic gene and protein expression, and greatly improved healing potential in a rat model of forearm critical bone defect [22]. A study on the combined use of metformin and alendronate showed that the alendronate use alone can increase serum GLP-1 levels significantly and the use of metformin alone can improve bone microstructure like Tb.Sp and Tb.N of the spinal in the control group. Consequently, metformin and alendronate in combination can improve the progress of glucose metabolism and bone metabolism such as lowering blood glucose levels, increasing glucose tolerance, increasing insulin sensitivity, and reducing bone loss than the control group, however, they do not appear to act in a clearly synergistic manner in their combined use [23].

3. Investigations on Clinical Settings

A large cohort study of Chinese patients with T2DM including 11,458 T2DM patients aged no less than 40 years showed that the overall prevalence of osteopenia and osteoporosis was 37.4% and 10.3% respectively, and was lower in metformin-treated patients (34.6% vs. 38.3% and 7.1% vs. 11.3%, both $p < 0.001$) [24]. Patients who had older age, a lower BMI, and estimated glomerular filtration rate (eGFR), had more osteoporosis, a lower BMD and T-score at the femoral neck (FN), lumbar spine (LS), and total hip (TH) [17]. Metformin use and the male sex were associated with a higher BMD. Metformin treatment was also independently associated with higher T-score at LS, FN and TH (b = 0.120, 0.082 and 0.108; all $p < 0.001$), and lower odds ratio (OR) of osteoporosis (OR = 0.779, 95%CI: 0.648–0.937, $p = 0.008$) or low BMD (OR = 0.834, 95%CI: 0.752–0.925, $p = 0.001$) [24]. However, when analyzed by sex, this association of a lower OR for osteoporosis with metformin was only significant in women. (OR = 0.775, 95%CI:0.633–0.948; $p = 0.013$) [24]. Consequently, metformin treatment was associated with a lower OR of osteoporosis and a higher T-score, particularly in the female population, regardless of age, BMI, and eGFR [24].

Currently, a prospective study with enrollments of 142 patients with T2DM treated with metformin or metformin plus a-glucosidase inhibitors was conducted in China. Their results showed that patients with metformin plus a-glucosidase inhibitors were associated with significantly lower levels of 2-h postprandial blood glucose (2hPG), hemoglobin A1c (HbA1c), fasting plasma glucose (FPG), and homeostasis model assessment-insulin resistance (HOMA-IR) vs. metformin alone ($p < 0.05$) after 12 weeks treatment [25]. The BMD index was correlated with IGF-1R positively and with vascular endothelial growth factor (VEGF) and endothelin negatively after treatment in both groups [25]. Metformin plus a-glucosidase inhibitors can effectively control blood glucose and reduce HOMA-IR in patients with primary T2DM, however, a large sample study was essential to predict osteoporosis development in T2DM patients [25].

A study about the effect of metformin on primary bone cancer risk conducted by Taiwan's National Health Insurance showed that the incidence rates were 10.56 and 12.90 per 100,000 person-years for 453,532 metformin initiators and 220,000 non-metformin initiators respectively, and the hazard ratio between initiators and non-initiators was 0.830 ($p = 0.0551$) in the intention-to-treat analysis. Additionally, the incidence rates were 7.58 and 11.77 per 100,000 person-years, respectively, and the risk ratio was 0.615 ($p = 0.0005$) in the per-protocol analysis [26]. In addition, metformin treatment in patients with excess endogenous glucocorticoid showed potential protective effects by reducing bone resorption, thereby reducing the undesirable side effects of glucocorticoid treatment [27].

In addition, one cannot ignore the side effects of metformin when it is applied in clinical settings. The most common adverse effect of metformin is gastrointestinal irritation, including diarrhea, cramps, nausea, vomiting, and increased flatulence; metformin is more commonly associated with gastrointestinal adverse effects than most other antidiabetic medications [28]. The most serious potential adverse effect of metformin is lactic acidosis; this complication is rare, and the vast majority of these cases seem to be related to conditions such as impaired liver or kidney function, rather than to the metformin itself [29]. Metformin is not approved for use in those with severe kidney disease, but may still be used at lower doses in those with kidney problems [30]. Lactic acidosis almost never occurs with metformin exposure during routine medical care [31]. Rates of metformin-associated lactic acidosis are about nine per 100,000 persons/year, which is similar to the background rate of lactic acidosis in the general population [32]. A systematic review concluded no data exists to definitively link metformin to lactic acidosis [33]. The risk of metformin-associated lactic acidosis is also increased by a massive overdose of metformin, although even quite large doses are often not fatal [34].

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