Review Article

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A Mini Review of the Literatures Regarding the Investigation of the Efficacy of the Dosage Forms Containing Metformin (MET) for Use in the Treatment of Periodontitis

Periodontitis Tedavisinde Kullanılmak üzere Metformin (MET) içeren İlaç Şekillerinin Etkililiğinin Araştırılmasına İlişkin Literatürlerin Kısa Bir Derlemesi

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ABSTRACT

The main objective of this review is to perform a review of the literature regarding the investigation of the efficacy of the dosage forms (gel and film for local application and nanoparticles for systemic administration) containing metformin (MET) for use in the treatment of periodontitis. The animal study or randomized clinical trials associated with the effects of the dosage forms on the treatment of periodontitis along with/without diabetes were included in this review. Periodontitis includes a wide range of inflammatory conditions. In recent studies, the effects of MET on inflammation, oxidative stress, or bone formation were evaluated in vitro/in vivo. These studies reported that MET decreases inflammatory response, oxidative stress, probing pocket depths (PPD), intrabony defect (IBD) depth, and bone loss. The clinical trials showed that the local delivery of MET using gel or film formulations has caused an improved response to therapy in patients with chronic periodontitis. There is only one study on the evaluation of the efficacy of MET-containing dosage forms in diabetic rats with periodontitis. Therefore, further studies should be conducted to assess the effects of MET-containing dosage forms for local or systemic administration on the treatment of periodontitis in the presence of diabetes.

Keywords: Clinical trials; diabetes; dosage forms; local or systemic application; metformin; periodontitis.

ÖZET

Bu derlemenin başlıca amacı, periodontitis tedavisinde kullanılmak üzere geliştirilen metformin (MET) içeren ilaç şakillerinin (lokal uygulama için jel ve film ve sistemik uygulama için nanopartiküller) etkinliğinin araştırılması ile ilgili literatürün derlenmesidir. İlaç şekillerinin diyabet varlığında/yokluğunda periodontitis tedavisi üzerindeki etkileri ile ilişkili hayvan çalışması veya randomize klinik çalışmalar bu incelemeye dahil edilmiştir. Periodontitis, çok çeşitli inflamatuar koşulları içerir. Son çalışmalarda MET'in inflamasyon, oksidatif stres veya kemik oluşumu üzerindeki etkileri in vitro/in vivo olarak değerlendirilmiştir. Bu çalışmalar, MET'in inflamatuar yanıtı, oksidatif stresi, cep derinliğini (CD), kemik içi defekti (KİD) derinliğini ve kemik kaybını azalttığını bildirmişlerdir. Klinik araş-

tırmalar, jel veya film formülasyonları kullanılarak MET'in lokal uygulamasıyla kronik periodontitisli hastalarda tedaviye daha iyi yanıt verdiğini göstermiştir. Periodontitisli diyabetik sıçanlarda MET içeren ilaç şekillerinin etkinliğinin değerlendirilmesi üzerine sadece bir çalışma bulunmuştur. Bu nedenle, lokal veya sistemik uygulama için MET içeren ilaç şekillerinin diyabet varlığında periodontitis tedavisi üzerindeki etkilerini değerlendirmek üzere daha fazla çalışma yapılmalıdır.

Anahtar Kelimeler: Diyabet; ilaç şekilleri; klinik çalışmalar; lokal veya sistemik uygulama; metformin; periodontitis.

1. Introduction

Periodontal disease (PD) includes a wide range of inflammatory conditions. In PD, there is a destruction of the supporting structures of the teeth (the gingiva, periodontal ligament and bone) by bacterial accumulation and maturation on teeth. This situation may cause tooth loss and may contribute to systemic inflammation [1-3]. Imbalance between osteoclast and osteoblast activities by bacterial products and inflammatory cytokines [interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) etc.] is the main cause of inflammation-induced bone loss in PD [4]. Osteoclastogenesis is a multi-step process including M-CSF (macrophage colony stimulating factor)-mediated osteoclast precursor proliferation, RANKL (receptor activator of NFκB ligand)-mediated osteoclast differentiation, osteoclast progenitor commitment. The RANK signaling pathway was influenced and modified by the cytokines such as IL-1, TNF-α and other factors, which are immune related and indicate the close relationship between the immune system, inflammatory processes and osteoclasts [5-7]. The use of inhibitors of IL-1 and TNF-α prevents the increase in bone resorption [8]. Besides, AMP-activated protein kinase (AMPK), plays an important role in regulating systemic energy homeostasis, osteoblast differentiation and bone development. It is a potential target for the intervention of some disease such as cancer and metabolic disorders [9-11]. AMPK is a primary mediator of the effects of many hormones in glucose metabolism [11]. Some studies reveal the importance of AMPK for osteoclastogenesis and show that AMPKα1 as a negative feedback regulator of RANKL inhibits the RANK signaling [10, 11]. Therefore, the deletion of AMPK in osteoblasts increases RANKL expression and consequently enhances bone turnover [9].

Diabetes mellitus (DM) is associated to several oral diseases (etc. oral bacterial and fungal infections, PD, taste disfuction) [2]. PD and DM are both highly prevalent diseases and there is a bidirectional relationship between DM and periodontitis. Chronic inflammation has been thought to be an important mechanism in the pathophysiology of type 2 diabetes. One of the causes of systemic inflammation is periodontitis. It was determined that routine oral health assessment and treatment of periodontitis might be important for effective control of Type 2 DM [12]. Epidemiological studies have shown that DM is a major risk factor for PD, especially if glycaemic control is insufficient. Permanent hyperglycaemia leading to excessive immune-inflammatory responses that are induced by bacteries on teeth is likely responsible for the high risk and severity of PD in patients with DM [4]. The risk of periodotitis in diabetic patients is three times higher than in those without diabetes [2, 13]. Hyperglycemia leads to an increase in the concentration of glucose in the saliva and an increase in the outflow of gingival crevicular fluid, resulting in the proliferation of bacteria in the oral cavity. Hyperglycemia has an indirect effect that stimulates cells of the immune system to secrete inflammatory cytokines. In periodontal pockets, increased proinflammatory mediator levels cause osteoclastic destruction. In poorly controlled diabetics, impaired immune response and lower resistance to infections and diabetic microangiopathy contribute to the development of periodontitis. Non-enzymatic glycation and oxidation of collagen fibers in the supporting periodontal ligaments are induced by continuous exposure to aldose sugars. As a result of glycation, collagen solubility decreases and the degradation of connective tissues increases and consequently, both connective tissue and bone degredade more rapidly [13,14]. DM is a chronic, serious, and complex disease characterized by a group of physiological dysfunctions such as inadequate insulin secretion, insulin resistance, or excessive glucagon secretion [15, 16]. In worldwide (especially, in middle- and low-income countries) approximately 422 million people have DM, which is one of the leading causes of death [17]. It is a major cause of kidney failure, stroke, heart attacks, blindness, and lower

limb amputation. There are two main types of DM [17]. In Type 1 DM, there is a destruction of pancreatic beta-cells. It is an autoimmune disorder. On the other hand, Type 2 DM, which is a multifactorial disease involving genetic and environmental factors, is much more common than Type 1 DM. It is characterized by a combination of pancreatic β-cell dysfunction, insulin resistance, and chronic inflammation [15, 18]. Type 2 DM has become a major global health problem. The number of patients with Type 2 DM is estimated to rise to 592 million by 2035. The patient with Type 2 DM needs a continuous medical care and self-management for the control of high glucose level. And also, the multifactorial risk reduction strategies is necessary to normalize blood glucose levels, blood pressure and lipid profiles to minimize/prevent the acute and long-term microvascular and macrovascular complications related to the Type 2 DM. Antidiabetic medications, especially in combination, is necessary to achieve the long-term glycaemic control in Type 2 DM [18].

Metformin (MET) is a biguanide derivative most commonly prescribed for the treatment of Type 2 DM. MET as an antihyperglycemic agent was approved in UK in 1958 and USA in 1995. The major effect of MET as an antihyperglycemic agent is to decrease hepatic glucose output. In addition, MET decreases the glucose absorption in the small intestine, lowers the plasma free fatty acid concentrations, and increases insulin-mediated glucose utilization in peripheral tissues such as liver and muscle [19].

In recent studies, the effects of MET on inflammation, oxidative stress [4, 20, 21] or on the growth and differentiation of osteoblast-like cells [22] or on alveolar bone loss [4, 23, 24] or on bone formation through activation of AMP-activated protein kinase (AMPK) [25] were evaluated in vitro or in vivo.

Cortizo et al. [22] evaluated in vitro the effects of MET (25-500 μM) on the growth and differentiation of osteoblast-like cells (UMR106: rat osteosarcoma cells; MC3T3E1: mouse calvaria-derived cells). In this study, a dose-dependent increase of cell proliferation was observed after 24 h incubation and it was reported that MET promoted osteoblastic differentiation and increased type-I collagen production in these cell lines. It also stimulated alkaline phosphatase activity and markedly enhanced the formation of nodules of mineralization in MC3T3E1 cells.

Bak et al. [23] assessed the effects of MET on alveolar bone loss in rat with ligature-induced periodontitis (Rats were intraperitoneally injected once per day for 10 days with saline or MET (10 mg/kg body weight) and also on osteoblast, osteoclast, and adipocyte differentiation using cell lines (MC3T3-E1, cocultures of mouse bone marrow cells and calvaria-derived osteoblasts, and Mouse 3T3-L1 preadipocytes cells). It was found that MET caused an important reduction in alveolar bone loss in rat with ligature-induced periodontitis. However, metformin has been shown to have no effect on osteoclast formation and adipocyte differentiation. As for osteoblast differentiation, MET nearly doubled the mineralization of MC3T3-E1 cells.

In addition, the effects of MET on biochemical, anthropometric and pro-inflammatory markers in obese patients with Type 2 DM were evaluated by Andrews et al. [20]. In these patients treated with MET, lower levels of C reactive protein (hsCRP) and lower mRNA relative abundance of TNF-α and Toll-Like Receptor 2/4 (TLR 2/4) were obtained.

In another study, the anti-inflammatory effects and mechanism of MET in intestinal inflammation using COLO205 (human, colon, colorectal adenocarcinoma) stimulated with TNF-α were investigated. MET significantly inhibited IL-8 induction in these cells and also attenuated inhibitor of kappaB (IκB) phosphorylation and transcription factor nuclear factor-kappaB (NF-κB) DNA-binding activity. It was reported that MET might be a potential therapeutic agent for the treatment of inflammatory bowel disease [21].

On the other hand, de AurauÂ jo et al. [4] evaluated its effects on the treatment of periodontitis after the systemic application of MET. They investigated the effects of MET (50 mg/kg-200 mg/kg) administered orally on oxidative stress, inflammation, and bone loss in a rat model of ligature-induced periodontitis. That found that the low concentration of MET (50 mg/kg) significantly reduced concentrations of IL-1β, TNF-α, and malondialdehyde. Consequently, the oxidative stress, inflammatory response, and bone loss in rats with ligature-induced periodontitis were reduced by MET.

In addition, the effects of MET on bone formation through activation of AMPK was studied by Kanazawa et al. [25]. They investigated in vitro the effects of MET on the differentiation and mineralization of osteoblastic cells and intracellular signal transduction. They determined that 50 μM of MET signifi-

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cantly increased collagen-I and osteocalcin mRNA expression and also induced endothelial nitric oxide synthase (eNOS) and bone morphogenetic protein-2 (BMP-2) expressions. It also activated AMPK in dose- and time-dependent manners, stimulated alkaline phosphatase activity, and enhanced cell mineralization. The activation of the AMPK signaling pathway is important to induce the differentiation and mineralization of osteoblasts by MET. Thus, they reported that MET might be beneficial for osteoporosis by promoting bone formation as well as diabetes.

In another study, the effects of MET on alveolar bone resorption and on the ratio of receptor activator of RANKL/osteoprotegerin (RANKL/OPG) in rats were evaluated. MET (40 mg/kg) was administered as daily intramuscular injections to rats. MET inhibits the periapical lesions possibly by reducing the RANKL/OPG ratio, and consequently, the number of osteoclasts and bone resorption sites are reduced [24].

Some studies have indicated that metformin has different potential indications as a cardiovascular protective agent, a neuroprotective agent, an anticancer agent, or a potential agent for polycystic ovary syndrome, osteoarthritis, and periodontitis. In this review, we summarized the efficacy of MET in the treatment of periodontitis and the efficacy of MET-containing dosage forms in diabetic subjects with periodontitis, which, unfortunately, there are not many studies on the second subject.

2. The Evaluation of The Effects of MET-Containing Dosage Forms on The Treatment of Patients with Periodontitis or Diabetic Animals with Periodontitis

Some formulations (gel, film and PLGA nanoparticles) containing MET have been developed for use in the treatment of PD and evaluated in vitro and/or in vivo. More studies on this subject have been conducted on a gel containing metformin for local application. Furthermore, randomized, controlled clinical trials [26-33] were performed for the investigation of the effectiveness of MET containing gel formulations or open flap debridement (OFD) with platelet-rich fibrin (PRF) plus MET containing-gel formulation or MET containing-gel formulation with plasma rich in growth factor (PRGF) or MET containing-film in the treatment of patients with chronic PD. Currently, there are several Phase III clinical trials to evaluate the effects of gel formulation containing MET (MET-gel) administered locally in the treatment of periodontitis [34]. In one of these studies, the efficacy of MET-gel (1%) in treatment of moderate and severe periodontitis was evaluated. In the other four studies, the efficacy of locally delivered MET-gel (1%) in treatment of in chronic periodontitis subjects was compared with alendronate gel (1%) or a gel formulation containing simvastatin (%1.2) or a gel formulation containing aloe vera or a gel formulation containing rosuvastatin (1.2%). In the other two studies, the efficacy of MET-gel (1%) in adjunct to scaling and root planing (SRP) or the efficacy of Platelet rich fibrin (PRF) with MET-gel (1%) in treatment of Class II furcation defects was determined [34].

Pradeep et al. [26] used MET-gel prepared using the method described by Mohapatra et al. [35] and some ingredients (gellan gum as hydrophilic polymer, mannitol and sucralose as co-solutes, citric acid and sodium citrate as buffering agents, methylparaben and propylparaben as preservatives). Firstly, the dispersion of gellan gum in distilled water was prepared and stirred for 20 min (at 95 ºC). After the adding of mannitol to the continuously stirred dispersion at above 80 ºC. Then, the different concentrations of MET (0.5% or 1% or 1.5%), sucralose, citric acid, methylparaben, propylparaben, sodium citrate were respectively added to the mixture of gellan gum and mannitol. Later, gel was formed by cooling the prepared mixture to room temperature [26]. When Mohapatra et al.'s study [35] on the preparation of oral soft gel containing MET is examined, it was observed that the MET-gel formulation prepared as described above was evaluated in terms of appearance, pH, viscosity, drug content, in vitro drug release, short-term stability at two different temperatures for four weeks, and also syneresis which is a problem associated with low acylated gellan gum gels and means contraction of gel and separation of water from the gel. It had been stated that UV spectrophotometer was used for the determination of active substance in both drug content and in vitro release studies. Besides, in the short-term stability study, the prepared gels were evaluated only in terms of viscosity, pH and appearance at 0-8 ºC and room temperature. However, the chemical stability of MET was not evaluated in the stability study. They reported only that the precipitation of MET in the gel formulation and syneresis

were not observed at both temperatures. And also, they found that the pH of the formulated gels were in the range of 5.78-5.86 with help of citric acid and sodium citrate for four weeks and reported that MET in aqueous phase is stable in the pH range of 4 and 9. However, the effects of the high temperature values used during the preparation of gel on the stability of MET were not evaluated. Furthermore, long-term stability studies for MET-gel formulations were not performed in this study [35]. SRP is a gold standard treatment for some patients with severe periodontitis. However, recently, various therapeutic approaches combined with SRP have been suggested to prevent the need for periodontal surgical procedures and to improve the results of SRP. The therapeutic approaches include the topical application of chlorhexidine to avoid bacterial translocation, the enhancement of instrument tip designs, laser use and the like [36]. The locally use of antimicrobial agents as a adjuvants for periodontal therapy cause a reduction of 0.47 mm for PDD [37].

For similar purpose, Pradeep et al. [26] injected the prepared MET-gel (10 μL) into the periodontal pockets using a syringe to determine the efficacy of MET-gel at different concentration (0.5%, 1%, and 1.5%) in adjunct to SRP for the treatment of intrabony defects in patients with chronic periodontitis [Thirty-seven of fortyone patients (21 females and 20 males; aged 30-50 years) diagnosed with chronic periodontitis completed this study]. They recorded some clinical parameters such as reductions in probing pocket depths (PPD) modified at baseline, 3 and 6 months. Besides, intrabony defect (IBD) depth as a radiographic parameter was recorded at baseline and 6 months [26]. It was obtained that there was a significant reduction in PPD (at 3 and 6 months) and IBD depth (at 6 month) in the MET-gel-treated groups as an adjunct to SRP compared to the blank-gel (without MET)-treated groups as an adjunct to SRP. They reported that especially, 1% MET-gel provided an optimum clinical benefit as it caused the greatest reductions in these clinical parameters. As a result of this study, the local application of MET-gel (especially, at 1% of concentration) combined with SRP was found to be effective for the treatment of intrabony defects in patients with chronic periodontitis [26].

In addition, Pradeep et al. [28] performed a further study to examine the efficacy of 1% MET-gel as an adjunct to SRP in the treatment of IBD in more patients (27 females and 38 males, aged of 25–50 years) with chronic periodontitis. The SRP was applied to all patients with chronic periodontitis. Later, 1% MET-gel or blank gel were applied into the periodontal pockets usinge a syringe. In this study, it was determined that there are significantly greater PD reduction and clinical attachment level (CAL) gain in patients treated with 1% MET-gel at 3 months and 6 months than those in patients treated with blank gel. And also, in patients treated with 1% MET-gel, significantly greater defect depth reduction (about 27 %) was observed at 6 months compared to that (about 5 %) in patient treated with blank gel.

In another study performed by Pradeep et al., [27] the efficacy of OFD combined with the application of Platelet-rich fibrin and 1% MET-gel in the treatment of IBD in patients with chronic periodontitis. OFD is a widely used procedure for the treatment of deep periodontal pockets and horizontal bone loss. However, this procedure can also result in significant gingival recession [38]. Besides, PRF consists of a leukocyte-platelet-rich fibrin matrix and acts as a biodegradable scaffold including cytokines, platelets, and stem cells. It has a sustained release of growth factors (such as platelet-derived growth factor and transforming growth factor 1b) in a period between 1 and 4 weeks. PRF promotes neovascularization and also can serve as a vehicle in carrying cells involved in tissue regeneration. Thus, PRF is a biomaterial with a high potential for bone and soft tissue [39]. Sharma and Pradeep showed in their previous study that better results (in terms of PPD reduction, and bone fill) in the treatment of IBD were obtained for the patients with chronic periodontitis treated with PRF with OFD compared to the patients treated with the only OFD [40].

In this study, Pradeep et al. [27] used the combination of OFD, PRF and MET-gel formulation prepared as described above. Firstly, the SRP was applied to the patients with moderate to severe chronic periodontitis (males and females with aged 30 to 50 years). After SRP application, the patients were treated with OFD or OFD plus PRF or OFD plus 1% MET-gel (10 μL) or OFD+PRF plus 1% MET-gel (10 μL). PRF and/or 1% MET-gel inserted into the IBD. Clinical parameters such as PPD, relative attachment level (RAL) and also radiographic IBD depth reduction were evaluated at baseline and 9 months. They reported that MET in IBD peaked at 2 hours after administration and was released from the gel for about

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4 weeks. A significantly greater PD reduction, IBD depth reduction and also RAL gain was observed in patients treated with OFD plus PRF or OFD plus 1% MET-gel or OFD+PRF plus 1% MET-gel compared to the obtained results in patient treated with OFD at 9 months. However, among all the groups, the most meaningful results were obtained for the group treated with OFD+PRF plus 1% MET-gel compared to the other groups.

Furthermore, the same group evaluated the efficacy of 1% MET-gel as an adjunct to SRP in the smokers patients with chronic periodontitis [29]. In this study, similar results were obtained with the previous studies in terms of PPD, CAL gain and IBD depth reduction in patients treated with MET-gel compared to those in patients treated with blank gel. 1% MET-gel along with SRP provided a significant improvement in clinical and radiologic parameters.

Besides, the effectiveness of MET (1%) and Aloe vera gel as an adjunct to SRP in the treatment of intrabony defects in patients (90 volunteers) with chronic periodontitis was evaluated after local administration [30]. The CAL gain, the mean PPD reduction, and the percentage of bone fill were higher in the patients treated with SRP plus MET (1%) and Aloe vera gel than those in the patients treated with SRP plus blank gel.

In addition, Grace and Sankari [41] investigated the efficacy of 1% MET-gel as an adjunct to SRP in the treatment of patients with chronic periodontitis. This study was conducted on a small group of 16 patients with chronic periodontitis (8 patients treated with MET-gel, the other 8 patients treated with blank gel). Clinical parameters [gingival index (GI), PPD and CAL] were evaluated at baseline and one month. They found that MET has an effect on the periodontal tissues of patients treated with MET-gel but there was no statistically significant differences between the clinical parameters in patients treated with MET-gel or blank gel. On the other hand, the short-term follow-up and studying with fewer patients are drawbacks of this study.

In another study, gel formulation contaning MET was prepared using Carbopol, preservatives (methyl paraben and propyl paraben) and glycerine. Furthermore, the efficacy of this formulation and plasma rich in growth factor (PRGF) was evaluated in the treatment of intrabony periodontal defects at baseline, 3 and 6 months. In the randomized clinical trial was performed in 8 patients (age ranged from 25 to 45 years; Group 1: SRP+debridement only, Group 2: SRP+debridement+1%MET-gel, Group 3: SRP+PRGF and Group 4: SRP+1% MET-gel+PRGF) with moderate chronic periodontitis. In all the groups, it was observed improvement in the clinical parameters (GI, PPD and CAL) of patients at 6 months but there was no a statistically significant difference among the clinical parameters of all the groups. However, when the radiographic changes among the groups were compared, there was obtained a statistically significant difference for patients treated with SRP+1% MET-gel+PRGF. However, among the other groups, there were no statistically significant differences [32].

Kotry et al. [33] assessed the effect of the mucoadhesive and multiple layer film containing MET (MET-film) on the patients with moderate/severe chronic periodontitis. The multiple layer mucoadhesive film were prepared using double casting followed by compression method as described in the previous study of the same group [42]. In brief, the aqueous solution of thiolated sodium alginate (T-SALG) put into a glass petri dish (5 cm in diameter) and dried at room temperature overnight. Later, to prepare the second layer, the solution of MET and SALG or carboxy methyl cellulose sodium (CMC) put on the top of the first layer and again dried in an incubator at 37 ºC (for 12 h). For the third layer of the film, the dried T-SALG film was mounted onto the film with little hydration and by compression with a roll. However, in these studies, the MET-film was not evaluated in terms of short- and long-term stability. After the preparation of the MET-film, SRP was performed in 20 patients (an age range from 36-55) with moderate –severe chronic periodontitis and the patients were divided two groups; Group I was treated with placebo and Group II was treated with MET-film. Clinical parameters were recorded at baseline, 3 and 6 months after application. Furthermore, IBD depth and bone density (BD) were evaluated radiographically at baseline and 6 months. PD reduction, IBD depth reduction, CAL gain and increase in BD in patients treated with MET-film were significantly higher than those in patients treated with placebo [33].

Pereira et al. [11] prepared MET HCl-loaded PLGA nanoparticles and evaluated the effect of MET-PLGA NPs on a ligature-induced periodontitis model in rats with diabetes. In ligature-induced periodontitis model, the expression of proinflammatory genes in

the liver and adipose tissues were up-regulated and also insulin resistance, along with a mild chronic inflammation was accelerated [43]. MET-NPs was prepared using the double emulsion solvent diffusion method. The aqueous solution of polyvinyl alcohol containing MET in was added to the PLGA solution in dichloromethane and homogenized with a probe-tip sonicator for 1 min. To obtain water/oil/water emulsion, the first emulsion was added to the aqueous solution of polyvinyl alcohol under magnetic stirring and dichlorometane was evaporated while stirring continued on the magnetic stirrer. To determine in vivo efficacy of MET-NPs with particle size about 457 nm and encapsulation efficiency of approximately 67%, MET (50 mg/kg/day or 100 mg/kg/day) or MET-NPs (equivalent to 10 mg/kg/day MET or 100 mg/kg/day MET) were administered orally to the rats with diabetes for 10 days and evaluated the effects of NPs on the levels of inflammatory markers such as TNF-α and IL-1β and bone loss. And also, they carried out quantitative RT-PCR analysis [11]. The application of pure MET or MET-NPs significantly reduced the level of IL-1β and TNF-α. However, inter groups, it was found that MET-NPs administered with low dose (equivalent to 10 mg/kg/day MET) was most effective in reducing bone loss (about a reduction of 0.49 cm), reducing the levels of TNF-α and IL-1β and also decreasing NF-κB p65, HMGB1 and TAK 1 gene expressions and increasing the gene expression of protein kinase AMPK. Furthermore, in the periodontal tissue samples of group treated with MET-NPs (equivalent to 10 mg/kg/day MET) was observed lower immunostaining for RANKL and cathepsin, but, an increase in immunostaining for osteocalcin compared to control group (untreated group of diabetic rats with periodontitis). Consequently, AMPK gene expression activated with MET-NPs led to stimulated osteocalcin expression and result in osteoblast deposition and bone formation. On the other hand, a decrease in RANKL expression results in low osteoclast activity [11]. Cathepsin K was expressed by the osteoclasts. It is one of the most important catalytic enzymes and degrades the Type I collagen of bone. On the other hand, osteocalcin is produced by osteoblasts and generally regarded as a bone formation marker [44]. RANKL and OPG are key molecules involved in the of activation and resorption osteoclast [45]. RANKL, which is expressed by osteoblasts, osteocytes, and stromal cells, is a very improtant factor for osteoclastogenesis. It binds RANK in osteoclast precursors and osteoclast formation is induced. On the other hand, OPG, which is produced by several cells such as osteoblasts, stromal cells, and gingival and periodontal fibroblasts [46] and its expression is regulated positively by some factors such as TGF-b, IL-1, TNF [47]. OPG binds to RANKL and prevents the RANK/RANKL interaction and results in inhibiting the osteoclastogenesis [46]. Thus, the RANK/RANK-L/OPG system is one of the primary regulators of the differentiation and function of osteoclast [44]. AMPK is crucial molecule in the mechanism of antidiabetic action of MET. MET has osteogenic effects on bone via AMPK and osteoblast specific transcription factor (Runx2). The stimulatory effects of AMPK signaling pathway on bone formation and bone mass is very important for bone physiology. AMPK may suppress RANKL-induced osteoclast formation [48]. Recently, it was shown that MET, which increases osteoblast proliferation and decreases osteoclast activity, has osteogenic effects on bone via AMPK and Runx2 [48]. It increases in OPG and decreases in RANKL expression and consequently reduces the number of osteoclasts and inhibits the alveolar bone resorption by lowering the RANKL/OPG ratio [11, 24]. The oral administration (100 mg/kg/day of MET application in drinking water for 2 weeks) of MET improves bone healing in rats by increasing osteoblast specific transcription factor (Runx2) and AMPK activation in a time-dependent manner [48, 49].

This review includes the studies on the efficacy of MET-dosage forms in the treatment of patients with PD or diabetic patients with PD, which present follow-up time ranging from 10 days to 9 months. It is known that the more pronounced changes in clinical parameters are observed in the initial months following periodontal treatment [37]. In these studies, after local or systemic use of the dosage forms containing MET, the reductions in PPD observed at initial 3 months, IBD depth observed at 9 months and gains in clinical attachment observed at initial 3 months. In addition, in these clinical studies, the local administration of MET-gel/-film formulations causes a reduction of in the range of 3.2-4 mm at 6 months [26, 28, 29, 33] and of 4.9 mm at 9 months [27] for PDD. The results revealed that the dosage forms containing MET have benificial effects on periodondal healing. The use of the dosage forms containing MET with the other periodontal treatment methods improves the periodontal parameters and may increase the

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efficacy of the periodontal treatment approaches. Furthermore, in the studies included in this review (except of the study on PLGA nanoparticles), the efficacy of the dosage forms containing MET was not compared to that of pure drug. Also, the long- or short-term stability studies for the dosage forms (especially in terms of the stability of MET in dosage forms) were incomplete or not carried out.

The clinical trials were generally performed by the same group. Besides, some studies have drawbacks such as the very short-term follow-up and studying with fewer patients. Therefore, additional findings to be obtained from clinical studies performed by different groups on the efficacy of dosage forms containing MET after local application are needed to increase validity of the data. Consequently, these clinical trials have demontrated that the local delivery of MET using gel or film formulations, along with SRP/OFD/PFD application, has caused an improved response to therapy in patients with chronic periodontitis. Especially, the local use of 1% MET-gel, by increasing the MET's concentration at the target site, decreasing dosage, significantly has improved clinical parameters and caused a reduction in IBD depth.

3. Conclusion

As can be seen, unfortunately, there are not many studies on the evaluation of the efficacy of MET-containing dosage forms in diabetic subjects with periodontitis. There is only one study, and in this study evaluating the effect of MET-PLGA NPs in diabetic and periodontitis rats, it was determined that the use of MET-PLGA NPs (equivalent to 10 mg/kg MET) significantly reduced inflamation and decreased bone loss. However, further in vitro and in vivo studies on MET-PLGA nanoparticles developed for the systemic administration of MET for the treatment of periodontitis in the presence of diabetes are needed to obtain additional data for healthy discussion and interpretation of the results. Besides, the effects of the dosage forms containing MET for local application on the treatment of the treatment of periodontitis in the presence of diabetes should be evaluated.

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