Original Article

Evaluation of Protective Effect of Metformin in Rats with Experimental Osteoarthritis

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Abstract

Objective: The objective of this study is to evaluate metformin proposed protective effect against the development of osteoarthritis (OA) in rats. **Materials and Methods:** A total of 40 male Sprague–Dawley rats were included, divided into four groups: negative control (n = 10), positive control (OA induced by monoiodoacetate [MIA]) (n = 10), metformin 200mg/kg group (n = 10) preinduction of OA, and metformin 200 mg/kg group (n = 10) postinduction of OA. Serum C-telopeptide type II collagen (CTX-II), inflammatory biomarkers were evaluated for each group. For data analysis, SPSS version 26.00 was used. **Results:** Metformin-treated groups showed a significant reduction in inflammatory biomarkers and CTX-II serum levels compared with OA group ($P < 0.05$). Pretreatment with 200 mg/kg metformin imparted extra cartilage protective effect and further decreased inflammatory cytokines compared to posttreated ($P < 0.05$). **Conclusion:** Metformin produced a beneficial protective effect in experimental OA in rats. It attenuates the inflammatory reactions progression by preventing the release of pro-inflammatory cytokines in rats with experimental OA. Furthermore, metformin reduced cartilage degradation evidenced by lowering CTX-II serum levels experimental OA in rats.

Keywords: C-telopeptide type II collagen-II, inflammatory biomarkers, metformin, osteoarthritis

Introduction

Osteoarthritis (OA) is a degenerative painful disorder that affects around 654 million individuals aging ≥40 years.[1] It is considered to be among the most expensive to treat conditions when total joint replacement is indicated, and in each year, around one million knee and hip replacements are performed.[2] OA is regarded as the third rapidly rising disorder associated with disability.[1] OA is primarily characterized by articular cartilage loss, ectopic bone formation, and synovial inflammation in the affected joint. This complex and chronic disease can affect any joint; yet, the most commonly affected joints are the: weight-bearing joints such as the knees, hips, feet, hands, neck, and lower back.[3] OA also disturbs bone remodeling and might lead to ectopic ossification or bone loss.[4,5] OA is an irreversible joint disease with no pharmacological management yet, except for symptomatic management, and when symptoms cannot be controlled, and mobility is affected, total joint replacement remains the best intervention.[3]

Metformin is a biguanide antidiabetic that is internationally used as an oral hypoglycemic agent, it is considered the drug of choice for type 2 diabetes according to the recent joint European–American clinical guidelines.[6] Exploring the molecular mechanisms developed over the years, the proposed application of metformin extended widely to variable conditions such as cancer, antiaging and polycystic ovary disease, although clinical evidence supporting the use of metformin in these diseases is insufficient.[7]

Low-grade chronic inflammation found to be a contribution in OA development and progression. Common inflammatory mediators, such as interleukin-1 (IL-1β) and tumor necrosis factor-alpha (TNF-α), and chemokines, were reported to have a contribution in the systemic inflammation in OA and nuclear factor kappa B (NF-κB) signaling is stimulated in chondrocytes and synovial cells. Furthermore, the innate inflammatory biomolcules were involved in OA, like damage associated

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molecular patterns, and complement. Recent findings further illustrated that systemic inflammation is able to modulate chondrocytes by inflammatory mediators for hypertrophic and catabolic activities by the NF-κB pathway.[8]

Recent evidence proposed a protective effect of metformin against OA development through the modulation of inflammatory reactions in rats.[9] However, the protective effect was not studied in most previous studies. Thus, due to its pleotropic effect, metformin might be effective in OA management. Hence, in the present study, we aim to investigate the possible protective effect of metformin against the development of OA in rats.

Materials and Methods

This research was accomplished in the College of Medicine, Department of Clinical Pharmacology and Therapeutics, Al-Mustansiriyah University, Baghdad/Iraq, from November 2021 to January 2022. This study was permitted and approved by the scientific Jury and Editorial Board in the College of Medicine, Al-Mustansiriyah University.

Animals

A 40 male Sprague–Dawley rats with weight range of 200–250 were purchased from the animal house of the Iraqi Cancer Research Center, Baghdad, Iraq. These rats were kept in well ventilated cages under 12-h/12-h light-dark cycle.

Induction of osteoarthritis

In this experiment, rats were anesthetized with isoflurane 2 mg/kg. Then, monoiodoacetate (MIA) 3mg/kg injected into the knee joint using 26G needle. After injection, the rats were kept under monitoring for 7 days following induction of OA. This method was done according to Feng *et al*. study.[9]

Study design

Forty rats were grouped evenly into four groups: the negative control group did not receive any agents (n = 10), positive control (group OA induced) received intra-articular MIA 3 mg/kg (n = 10), orally treated 200 mg/kg of Metformin 1 week preinduction (n = 10), and orally treated 200 mg/kg of metformin 1-week postinduction (n = 10). The duration of the study was 2 weeks. The experiment was done according to the guidelines for the use and care of laboratory animals.

Biochemical profile

At the end of the experiment, the animals were euthanized, and blood samples were collected and immediately centrifuged at 3000/rpm. The sera were stored in the proper conditions (at − 20° C) for analysis.

Inflammatory biomarkers, including

TNF-α, IL-2, IL-6, and IL-1β, were measured by enzyme linked immunosorbent assay (ELISA) kit methods (LABISKOMA, Inc. Korea). Serum C-telopeptide type II collagen (CTX-II) also was measured using the ELISA kit method (MyBioSource). Procedures of ELISA kits were performed according to manufacture protocol and instructions.

Data analyzed statistically by SPSS (Statistics for Window version 26.00, 2018, Armonk, NY, IBM, Corp, USA). The presented data were expressed as a mean ± standard deviation. One-way analysis of variance was used to detect the differences among groups. The level of significance was applied at $P < 0.05$.

Results

Inflammatory biomarkers (TNF-α, IL-1β, IL-6, and IL-2) were highly elevated in OA untreated group (positive control) relative to the negative control ($P < 0.01$). However, these inflammatory biomarkers reduced significantly in metformin treated groups as compared to the positive control ($P < 0.01$) [Table 1 and Figure 1].

After OA induction, CTX-II serum level increased in positive control up to 5.98 ± 1.05 ng/ml relative to 1.70 ± 0.84 ng/ml in the negative control group ($P = 0.0001$). In metformin-treated groups, CTX-II serum level was significantly reduced to 0.91 ± 0.32 ng/ml in the pretreated group and 2.22 ± 0.321 ng/ml in the posttreated group as compared to the positive control group ($P = 0.0001$) [Figure 2].

Discussion

The results of the current study demonstrated that metformin exerts an anti-inflammatory property when comparing inflammatory biomarkers levels (IL-1β, IL-2, IL-6, and TNF-α) versus the positive control group. It was previously documented in several studies that metformin acquires several nonglycemic effects.[7] Metformin has potent anti-inflammatory properties; when was tested against the inflammation caused by lipopolysaccharide in middle ear epithelial cell line.[10] Furthermore, in experimental arthritis, metformin dose-dependent use decreased TNF- α and IL-6 through the activation of AMP-activated protein kinase (AMPK)[11] likewise: sepsis inflammatory cytokines (IL-6, IL-1β and TNF- α) produced by neutrophil and monocyte were reduced by metformin[12] and several other studies that all yielded the same finding.[13-17] In addition, metformin induced

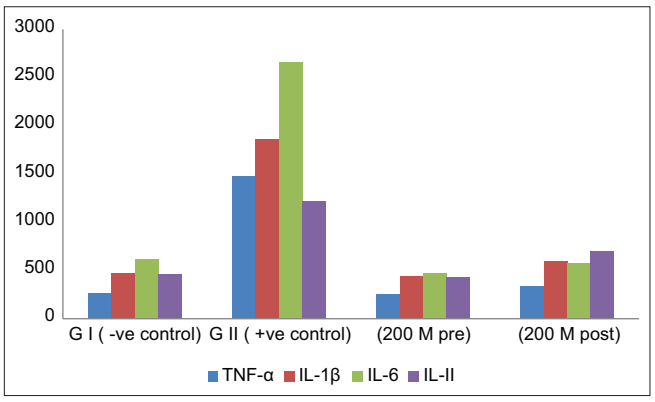

Figure 1: The protective effects of metformin on TNF-α, IL-1β, Il-6, and IL-Il serum level in rats with experimental OA. OA: Osteoarthritis, TNF-α: Tumor necrosis factor-alpha, IL: Interleukin

Table 1: Inflammatory biomarkers in rats with experimental osteoarthritis regarding pre- and posteffect of metformin

Biomarkers	Negative control	Positive control	Metformin 200 mg/kg	
			Preinduction	Postinduction
TNF-α (ng/ml)	240.86±12.83	390.86±14.56[1]	251.22±31.94	335.29±63.26
IL-1β (ng/ml)	470.66±19.83	864.93±13.66[1]	439.5296±102.96	597.1185±148.83
IL-2 (ng/ml)	488.84±22.07	952.05±29.99[1]	424.678±65.56	705.242±62
IL-6	674.93±21.18	994.31±29.33[1]	466.135±61.58	579.006±187.77

TNF-α: Tumor necrosis factor-alpha, IL: Interleukin

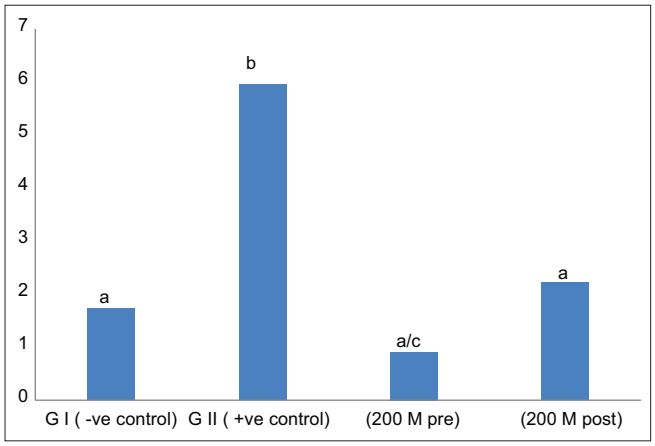

Figure 2: The protective effects of metformin on CTX-II serum level in rats with experimental OA. OA: Osteoarthritis, CTX-II: C- telopeptide type II collagen

expression of FOXP3 (forkhead box P3), a master regulator of Treg (regulatory T cells), which modulate inflammatory reactions and ameliorate the autoimmune disease severity in animal models[18] Proposed mechanism of metformin anti-inflammatory effect is not fully elucidated. However, and as AMPK is considered an emerging controller of the inflammatory process in OA;[19-21] Hence, metformin as a well-known AMPK modulator might improve OA through inhibiting mTOR and NF-κB pro-inflammatory signaling cascades, in addition to several other non-AMPK-dependent anti-inflammatory pathways that were also suggested.[22]

Regarding cartilage protective effect, CTX-II is the diagnostic biomarker for cartilage degradation in OA.[23] Measuring urinary level of CTX-II indicate disease severity and matrix integrity.[22] Results obtained of the present study confirmed that metformin use had lowered serum CTX-II levels significantly, with values comparable to the normal group. Recent review also demonstrated metformin effect against knee OA, evidenced by lowering serum CTX-II level in preclinical and human researches.[24] metformin through modulating AMPK activity enhances collagen II production and suppress MMP production by chondrocyte thus imparting an anabolic and anticatabolic effect.[25,26]

Pretreatment with metformin significantly lowered CTX-II serum level indicating protective effect against cartilage degradation. IL-II also significantly reduced; other measured inflammatory biomarkers were also reduced when metformin was pretreated, although not to the significant level. The limitation of this study is the small sample size.

Conclusion

Metformin imparted beneficial protective effects against OA development in experimental rats. Metformin weakens the progression of inflammatory reactions by obstructing the release of pro-inflammatory cytokines in rats with experimental OA. In addition, metformin capable of reducing cartilage degradation as approved by decreasing serum levels of CTX-II in rats with experimental OA.

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Conflicts of interest

There are no conflicts of interest.

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