RESEARCH ARTICLE

Evaluation of the protective dose-dependent effect of metformin for induced osteoarthritis in rats

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Abstract

Objective: To evaluate the potential protective effect of metformin against osteoarthritis development in rats.

Method: The experimental study was conducted at the Iraqi Centre for Cancer Research and Medical Genetics, Mustansiriyah University, Baghdad, Iraq, from December 2021 to February 2022, and comprised male Sprague-Dawley mice who were divided into 5 equal groups: negative control group, osteoarthritis group subjected to monoiodoacetate induction, positive control group treated with celecoxib 30mg/kg, metformin 100mg/kg group, and metformin 200mg/kg group. Body mass index, inflammatory biomarkers, and serum C-terminal cross-linked telopeptide of type II collagen levels were noted for all the animals. Data was analysed using SPSS 24.

Results: Of the 35 mice, 7(20%) were in each of the 5 groups. Compared to the osteoarthritis group, metformin-treated mice showed significantly reduced body mass index, inflammatory biomarker levels, and blood levels of C-terminal cross-linked telopeptide of type II collagen (p=0.05). Metformin 200mg/kg treatment had more beneficial effects than 100mg/kg dose on inflammatory biomarkers and serum C-terminal cross-linked telopeptide of type II collagen (p=0.05).

Conclusions: A beneficial protective effect against the onset of osteoarthritis was produced by metformin in a dose-dependent way. Additionally, metformin could lessen cartilage damage as demonstrated by a decrease in the serum levels of C-terminal cross-linked telopeptide of type II collagen in the osteoarthritis group.

Key Words: Collagen, Celecoxib, Osteoarthritis, Metformin, Cartilage

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Introduction

Osteoarthritis (OA) is a degenerative joint disease that results from the destruction of joint cartilage and associated bone[1]. OA is usually progressive, and, unlike inflammatory arthritis, internal organs are not affected[1]. Primary OA is idiopathic and mainly affects the knee and hip joints, which may develop due to hormonal changes in menopause[2]. However, secondary OA is caused primarily by different metabolic and inherited diseases, including obesity, trauma, haemochromatosis, Wilson disease, Ehlers-Danlos syndrome, and ligamentous disorders[3]. OA is commonly diagnosed by specific criteria that depend on the radiological and clinical manifestations[4]. Besides, management of OA is chiefly done by weight-loss, physiotherapy and the use of analgesic and anti-inflammatory agents[4]. Of note, different therapeutic strategies were tried to improve clinical outcomes in OA patients. In addition, intra-articular injection of hyaluronic acid and platelet-rich plasma was ineffective and produced placebo-like effects[5, 6]. Therefore, searching for OA management is mandatory nowadays.

Metformin (MTN) is a biguanide insulin-sensitising agent that inhibits hepatic gluconeogenesis and improves the insulin sensitivity of body tissues. MTN is regarded as a first-line agent in the management of type 2 diabetes mellitus (T2DM) patients[7]. Recent findings suggest that MTN has pleiotropic effects, including antiviral and anti-inflammatory effects through the modulation of pro-inflammation cytokines[8]. A recent experimental study illustrated the protective effect of MTN against the development of OA through the modulation of inflammatory reactions in mice[9]. However, the dose-dependent effect has generally not been evaluated.

The current study was planned to evaluate the dose-dependent effectiveness of MTN against the development of OA in mice.

Materials and Methods

The experimental study was conducted at the Iraqi Centre for Cancer Research and Medical Genetics, Mustansiriyah University, Baghdad, Iraq, from December 2021 to February 2022, and comprised male Sprague-Dawley mice weighing 200-250g. After approval from the ethics review board of the College of Medicine, Mustansiriyah University, Baghdad, Iraq, the animals were housed in

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sterile cages, 3 to a cage, with standard and suitable atmospheric conditions and under a 12-hour light/dark cycle. All mice had unlimited access to food and water. To ensure, all the rats were allowed to remain in the cages for a full week.

Each mouse was anaesthetised with the use of isoflurane 2mg/kg. Then, MIA 3mg/kg)Ambeed, United States), in sterile saline (0.9% sodium chloride [NaCl]) (PSI, Saudi Arabia), was injected into the knee joint pocket using 26G sterile syringes. The rats were monitored for 7 days post-induction[9].

The mice were divided into 5 equal groups: negative control group, OA group subjected to monoiodoacetate (MIA)) Ambeed, United States) induction, positive control group treated with celecoxib 30mg/kg (Pfizer, USA), MTN 100mg/kg group, and MTN 200mg/kg group (Merck serono, France). The procedure lasted 2 weeks.

A graduated tape was used to measure the naso-anal length of each mouse. The body weight was measured using a digital balance. The body mass index (BMI) was calculated using the standard equation[10].

At the end of the intervention, all the animals were sacrificed under general anaesthesia (GA) using isoflurane 2mg/kg)) Ambeed, United States), and blood samples were collected directly by cardiac puncture. The samples were allowed to coagulate at room temperature and then centrifuged at 3000rpm. The sera were stored at -20°C in a specific refrigerator till analysis.

Enzyme-linked immunosorbent assay (ELISA) techniques were used to measure inflammatory biomarkers such as tumour necrosis factor-alpha (TNF-α), interleukins (IL), IL-1, IL-2 and IL-6 (Labiskoma, Inc. South Korea). An ELISA kit (My BioSource, USA) was used to quantify serum C-terminal cross-linked telopeptide of type II collagen (CTX-II) in accordance with the manufacturer's instructions.

Data was analysed using SPSS. Data was expressed as mean and standard deviation (SD). In order to identify differences between unrelated groups, unpaired t-test was used. Also, the differences between unrelated groups were found using a one-way analysis of variance (ANOVA). P≤0.05 was taken as the level of significance.

Results

Of the 35 mice, 7(20%) were in each of the 5 groups. Compared to OA group, MTN-treated mice showed significantly reduced BMI (Table 1) and inflammatory biomarker levels (Table 2).

Table-1: Body mass index acorss the study groups.

Rats BMI	Before (n=35)	After (n=35)	Difference	95% CI	P
				0.0016-0.038	
Negative control	0.55±0.02	0.57±0.01	0.020	-0.048	0.03
OA group	0.57±0.01	0.54±0.02	-0.030	0.011	0.004
Positive control	0.57±0.02	0.55±0.01	-0.020	-0.038-	0.03
MTN 100 mg/kg	0.58±0.03	0.53±0.01	-0.050	0.001	0.001
MTN 200 mg/kg	0.58±0.01	0.51±0.02	-0.070	-0.076-0.024	0.0002
				-0.099-0.040	

Data expressed as mean ± Standard deviation. OA: Osteoarthritis, MTN: Metformin, CI: Confidence interval.

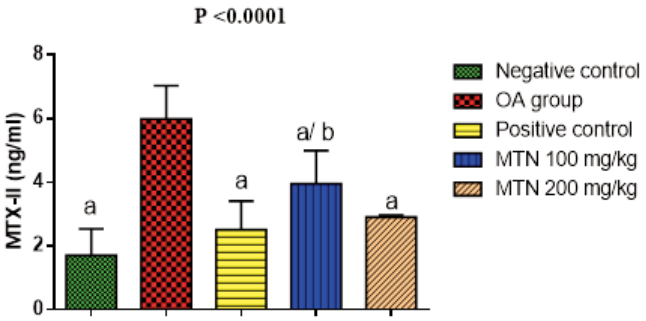

Figure: Dose-dependent effects of metformin (MTN) on CTX-II serum level across the study groups.
CTX-II: C-terminal cross-linked telopeptide of type II collagen, OA: Osteoarthritis, MTN: Metformin.a P<0.001 vs. OA group, b P<0.05 vs. positive group

Table-2: Inflammatory biomarkers across the study groups.

Biomarkers	Negative control	OA group	Positive control	MTN 100 mg/kg	MTN 200 mg/kg
TNF-α (ng/ml)	240.86±12.83	390.86±14.56!	280.05±13.11	277.11±10.22	260.44±12.01*#
IL-1β(ng/ml)	470.66±19.83	864.93±13.66!	560.71±10.33	740.71±22.04	590.31±12.18*#
IL-2(ng/ml)	488.84±22.07	952.05±29.99!	660.94±11.02	710.21±13.99	588.52±13.05*#
IL-6	674.93±21.18	994.31±29.33!	710.06±19.55	810.55±13.16	740.99±21.91*#

*P<0.01 vs positive control, #P<0.05 vs MTN 100 mg/kg, ! P<0.01 vs negative control. OA: Osteoarthritis, MTN: Metformin, TNF: Tumour necrosis factor, IL: Interleukin.

During OA induction, CTX-II serum level increased in the OA group to 5.98±1.05ng/ml compared to 1.70±0.84ng/ml in the negative control groups (p=0.0001). In the positive control group, CTX-II serum level reduced significantly to 2.50±0.91ng/ml compared to the OA group (p=0.0001). Regarding the differential effects of MTN therapy, CTX-II serum level reduced to 3.95±1.04ng/ml (p=0.01), and 2.90±0.05ng/ml (p=0.24) in rats treated with MTN 100mg/kg and MTN200mg/kg, respectively compared to the positive controls (Figure).

Discussion

The present study's findings illustrated that the use of MTN reduced body weight significantly compared to the positive controls. Different experimental and clinical studies have demonstrated that MTN therapy could decrease body weight, mainly in obese patients with T2DM[11,12]. The underlying mechanism of MTN in the reduction of body weight is related to the modulation of adipocytokines and inflammatory milieu[11,12]. Remarkably, MTN induces the expression of glucagon-like peptide-1 (GLP-1) through the modulation of gut microbiota[13]. In turn, increased circulating GLP-1 can decrease body weight through modulation of appetite[14]. Adams et al. found that liraglutide, a GLP-1 receptor agonist, inhibits appetite by suppressing glutaminergic neurons[14]. Recently, MTN therapy has exerted an anorexic effect through induction expression and release of growth differentiation factor-15 (GDF-15), which triggers taste aversion through inhibition of the hypothalamic feeding centre[15].

Besides, the present study found that MTN therapy reduced inflammatory biomarkers in mice with experimental OA in a dose-dependent manner. MTN monotherapy in 100mg/kg and 200mg/kg can significantly decrease IL-1β, IL-2, IL-6 and TNF-α compared to positive controls. Of note, MTN had potent anti-inflammatory effects. Afshari et al. reported that MTN therapy improved locomotor activity and neuropathic pain by inhibiting the release of pro-inflammatory cytokines[16]. MTN exerts local and systemic anti-inflammatory effects through the inhibition of inflammatory signalling pathways[17]. MTN inhibits the expression of nuclear factor kappa B (NF-κB) and the mechanistic target of rapamycin (mTOR) that are involved in the expression of pro-inflammatory cytokines[18]. Thus, MTN therapy, via its anti-inflammatory properties, may reduce the associated inflammation in OA. Type II collagen, also known as CTX-II, is regarded as a prognostic biomarker of OA[19]. Many recent studies have used urinary CTX-II as a crucial biomarker of disease severity and matrix degeneration in OA[20]. However, the current study measured serum CTX-II in a mice model with experimental OA. The findings confirmed that MTN had the ability to decrease CTX-II serum levels in a dose-dependent manner. a systematic review demonstrated that MTN was effective against knee OA, as evidenced by decreasing CTX-II serum level in preclinical and human studies[21]. The possible mechanism of MTN against the development of OA is not well elucidated. However, a recent study showed that MTN improved adenosine monophosphate-activated protein kinase (AMPK) expression in the chondrocytes with subsequent inhibition of matrix metalloproteinases (MMPs), enhancing the production of CTX-II[22]. In addition, a cohort study involving 968 T2DM patients with OA treated with MTN illustrated that such patients were less likely to undergo joint replacement surgery[23]. A prospective study showed that MTN therapy in obese patients with OA had a potential effect on the prevention of knee cartilage injury[24].

Limitations: The present study has several limitations, including small sample size and serial measurement of inflammatory biomarkers. The sample size was not calculated which could have affected the power of the study and the generalisability of the findings. Also, there was no estimation of urine CTX-II levels.

Conclusion

A beneficial protective effect against the onset of OA was produced by MTN in experimental mice in a dose-dependent manner. Additionally, MTN could lessen cartilage damage, as demonstrated by a decrease in the serum levels of CTX-II in mice with experimental OA.

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References

1. Abramoff B, Caldera FE. Osteoarthritis: Pathology, Diagnosis, and Treatment Options. Med Clin North Am 2020;104:293-311. doi: 10.1016/j.mcna.2019.10.007.
2. Briani RV, Ferreira AS, Pazzinatto MF, Pappas E, De Oliveira Silva D, Azevedo FM What interventions can improve quality of life or psychosocial factors of individuals with knee osteoarthritis? A systematic review with meta-analysis of primary outcomes from randomised controlled trials. Br J Sports Med 2018;52:1031-8. doi: 10.1136/bjsports-2017-098099.
3. De Lucia O, Murgo A, Pregnolato F, Pontikaki I, De Souza M, Sinelli A, et al. Hyaluronic Acid Injections in the Treatment of Osteoarthritis Secondary to Primary Inflammatory Rheumatic

Diseases: A Systematic Review and Qualitative Synthesis. Adv Ther 2020;37:1347-59. doi: 10.1007/s12325-020-01256-7.
4. Zhang Z, Huang C, Jiang Q, Zheng Y, Liu Y, Liu S, et al. Guidelines for the diagnosis and treatment of osteoarthritis in China (2019 edition). Ann Transl Med 2020;8:1213. doi: 10.21037/atm-20-4665.
5. Su K, Bai Y, Wang J, Zhang H, Liu H, Ma S. Comparison of hyaluronic acid and PRP intra-articular injection with combined intra-articular and intraosseous PRP injections to treat patients with knee osteoarthritis. Clin Rheumatol 2018;37:1341-50. doi: 10.1007/s10067-018-3985-6.
6. Belk JW, Kraeutler MJ, Houck DA, Goodrich JA, Dragoo JL, McCarty EC. Platelet-Rich Plasma Versus Hyaluronic Acid for Knee Osteoarthritis: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Am J Sports Med 2021;49:249-60. doi: 10.1177/0363546520909397.
7. Al-Kuraishy HM, Sami OM, Hussain NR, Al-Gareeb AI. Metformin and/or vildagliptin mitigate type II diabetes mellitus induced-oxidative stress: The intriguing effect. J Adv Pharm Technol Res 2020;11:142-7. doi: 10.4103/japtr.JAPTR_18_20.
8. Al-Kuraishy HM, Al-Gareeb AI, Alblihed M, Cruz-Martins N, Batiha GE. COVID-19 and Risk of Acute Ischemic Stroke and Acute Lung Injury in Patients With Type II Diabetes Mellitus: The Anti-inflammatory Role of Metformin. Front Med (Lausanne) 2021;8:644295. doi: 10.3389/fmed.2021.644295.
9. Feng X, Pan J, Li J, Zeng C, Qi W, Shao Y, et al. Metformin attenuates cartilage degeneration in an experimental osteoarthritis model by regulating AMPK/mTOR. Aging (Albany NY) 2020;12:1087-1103. doi: 10.18632/aging.102635.
10. Rasheed HA, Al-Naimi MS, Hussien NR, Al-Harchan NA, Al-Kuraishy HM, Al-Gareeb AI. New insight into the effect of lycopene on the oxidative stress in acute kidney injury. Int J Crit Illn Inj Sci 2020;10(Suppl 1):s11-6. doi: 10.4103/IJCIIS.IJCIIS_113_19.
11. Al-Kuraishy HM, Al-Gareeb AI, Waheed HJ, Al-Maiahy TJ. Differential effect of metformin and/or glyburide on apelin serum levels in patients with type 2 diabetes mellitus: Concepts and clinical practice. J Adv Pharm Technol Res 2018;9:80-6. doi: 10.4103/japtr.JAPTR_273_18.
12. Al-Kuraishy HM, Al-Gareeb AI, Shams HA, Al-Mamorri F. Endothelial dysfunction and inflammatory biomarkers as a response factor of concurrent coenzyme Q10 add-on metformin in patients with type 2 diabetes mellitus. J Lab Physicians 2019;11:317-22. doi: 10.4103/JLP.JLP_123_
13. Bahne E, Sun EWL, Young RL, Hansen M, Sonne DP, Hansen JS, et al. Metformin-induced glucagon-like peptide-1 secretion contributes to the actions of metformin in type 2 diabetes. JCI Insight 2018;3:e93936. doi: 10.1172/jci.insight.93936.
14. Adams JM, Pei H, Sandoval DA, Seeley RJ, Chang RB, Liberles SD, et al. Liraglutide Modulates Appetite and Body Weight Through Glucagon-Like Peptide 1 Receptor-Expressing Glutamatergic Neurons. Diabetes 2018;67:1538-48. doi: 10.2337/db17-1385.
15. Coll AP, Chen M, Taskar P, Rimmington D, Patel S, Tadross JA, et al. GDF15 mediates the effects of metformin on body weight and energy balance. Nature 2020;578:444-8. doi: 10.1038/s41586-019-1911-y.
16. Afshari K, Dehdashtian A, Haddadi NS, Haj-Mirzaian A, Iranmehr A, Ebrahimi MA, et al. Anti-inflammatory effects of Metformin improve the neuropathic pain and locomotor activity in spinal cord injured rats: introduction of an alternative therapy. Spinal Cord 2018;56:1032-41. doi: 10.1038/s41393-018-0168-x.
17. Kristófi R, Eriksson JW. Metformin as an anti-inflammatory agent: a short review. J Endocrinol 2021;251:R11-22. doi: 10.1530/JOE-21-0194.
18. Al-Kuraishy HM, Al-Gareeb AI, Al-Hussaniy HA, Al-Harcan NAH, Alexiou A, Batiha GE. Neutrophil Extracellular Traps (NETs) and Covid-19: A new frontiers for therapeutic modality. Int Immunopharmacol 2022;104:108516. doi: 10.1016/j.intimp.2021.108516.
19. Arunrukthavon P, Heebthamai D, Benchasiriluck P, Chaluay S, Chotanaphuti T, Khuangsirikul S. Can urinary CTX-II be a biomarker for knee osteoarthritis? Arthroplasty 2020;2:6. doi: 10.1186/s42836-020-0024-2.
20. Ok SM, Lee SM, Park HR, Jeong SH, Ko CC, Kim YI. Concentrations of CTX I, CTX II, DPD, and PYD in the urine as a biomarker for the diagnosis of temporomandibular joint osteoarthritis: A preliminary study. Cranio 2018;36:366-72. doi: 10.1080/08869634.2017.1361624.
21. Lim YZ, Wang Y, Estee M, Abidi J, Udaya Kumar M, Hussain SM, et al. Metformin as a potential disease-modifying drug in osteoarthritis: a systematic review of pre-clinical and human studies. Osteoarthritis Cartilage 2022;30:1434-42. doi: 10.1016/j.joca.2022.05.005.
22. Cai X, Yuan S, Zeng Y, Wang C, Yu N, Ding C. New Trends in Pharmacological Treatments for Osteoarthritis. Front Pharmacol 2021;12:645842. doi: 10.3389/fphar.2021.645842.
23. Lu CH, Chung CH, Lee CH, Hsieh CH, Hung YJ, Lin FH, et al. Combination COX-2 inhibitor and metformin attenuate rate of joint replacement in osteoarthritis with diabetes: A nationwide, retrospective, matched-cohort study in Taiwan. PLoS One 2018;13:e0191242. doi: 10.1371/journal.pone.0191242.
24. Wang Y, Hussain SM, Wluka AE, Lim YZ, Abram F, Pelletier JP, et al. Association between metformin use and disease progression in obese people with knee osteoarthritis: data from the Osteoarthritis Initiative-a prospective cohort study. Arthritis Res Ther 2019;21:127. doi: 10.1186/s13075-019-1915-x.