Alexandria Journal of Medicine (2016) xxx, xxx–xxx

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Alexandria Journal of Medicine

http://www.elsevier.com/locate/ajme

L-Carnitine ameliorates knee lesions in mono-iodoacetate induced osteoarthritis in rats

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Received 28 October 2015; revised 24 January 2016; accepted 7 March 2016

KEYWORDS

L-Carnitine;
Glucosamine;
MMP-13;
Osteoarthritis;
IL-1β;
COMP

Abstract *Objective:* To evaluate the chondroprotective effect of L-carnitine in relation to glucosamine sulfate and in an experimental model of osteoarthritis (OA).
Materials and methods: Thirty-two adult male Wister albino rats weighing 150–210 g were assigned randomly into 4 groups: 8 rats in each group, group I (control group), group II (MIA induced OA group), group III (MIA induced OA + glucosamine sulfate treated group), and group IV (MIA induced OA + L-carnitine treated group). Weight, knee diameter, and knee bend score were recorded on days 0, 1, 7, 14 and 28. On day 28 all animals were sacrificed. Synovial fluid of left knee was collected, and the interleukin-1β (IL-1β), Cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase-13 (MMP-13) levels were measured by ELISA. The knee joints were removed and stained with H&E for histological evaluation.
Results: The pathological abnormalities attributed to MIA induced arthritis was dramatically lowered in rats treated with glucosamine or L-carnitine. Synovial fluid levels of IL-1β, COMP and MMP-13 were increased in OA group, and significantly reduced with glucosamine or L-carnitine treated groups.
Conclusion: L-Carnitine has a potential chondroprotective effect in this animal model of OA.

1. Introduction

Osteoarthritis[1] is one of the most common forms of degenerative joint disease and a major cause of pain and disability affecting the aging population. Several factors including genetic susceptibility, obesity, injuries and inflammation of the joint have been long considered as important risk factors of the disease.[2]

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Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2016.03.002

Under normal conditions, a dynamic equilibrium between synthesis and degradation of extracellular matrix [ECM] components is maintained.[3] In osteoarthritic states, however, a disruption of matrix equilibrium leads to cartilage degradation, induction of oxidative states, and eventually, apoptosis of chondrocytes.[4] The triggering events and exact pathologic mechanism that result in cartilage loss and degradation are not completely understood. It has been suggested that inflammatory mediators such as cytokines [IL-1β, TNFα, IL-6, IL-8, and IL-17], chemokine and reactive oxygen species[5] have a primarily destructive impact on articular cartilage.[3] These inflammatory mediators lead to increase synthesis and release of matrix metalloproteinases [MMPs] and cartilage degradation.[6]

Analgesics and non-steroidal anti-inflammatory drugs [NSAIDs] are the main therapeutic treatment options for OA. Unfortunately, these medications are short-term and fail to adequately address pathophysiological and biochemical mechanisms involved with cartilage degeneration and the induction of pain in arthritic joints.[7] The search for effective treatment/dietary supplements able to slow the progression of disease seems warranted.

L-Carnitine [LC] [3-hydroxy-4-N-trimethylaminobutyrate], the bioactive form of carnitine, is an endogenous branched nonessential amino acid derivative that plays a critical role in energy production. It transports long-chain fatty acids from the cytoplasm to mitochondria so they can be oxidized to produce energy.[8] L-Carnitine bioavailability is 5–18%[9] and eliminated from the body mainly via urinary excretion.[10] L-Carnitine has been shown to offer a great therapeutic potential against several chronic conditions including cardiovascular, diabetes, neurodegenerative, and inflammatory diseases.[11] High dose can cause nausea, vomiting, abdominal cramps, diarrhea, a "fishy" body odor and rarely muscle weakness and seizures.[12]

Glucosamine [GA] naturally occurring 6-carbon amino sugar, normally found in the body.[13] The oral bioavailability of glucosamine is about 26%. It is distributed to liver, kidney and other tissues including articular cartilage.[14] The mechanism of action of glucosamine is unknown. Glucosamine was demonstrated to reverse the deleterious effects of IL-1β, nuclear factor-κB and prostaglandin E2.[15] Glucosamine appears to be safe, with possibility of causing allergic reaction and possibly increasing the risk of developing diabetes.[16]

Unilateral intra-articular [i.a.] injection of mono-iodoacetate[17], a chondrocyte glycolytic inhibitor, has been used to induce osteoarthritis-like changes in the articular cartilage of rodents. This minimally invasive model reproduces cartilage lesions with loss of proteoglycan matrix and functional joint impairment similar to human OA.[3]

In this study, knee bend test, histological examination of knee joints, in addition to synovial fluid levels of interleukin-1β [IL-1β], and matrix metalloproteinase-13 [MMP-13] were estimated to evaluate the effect of L-carnitine, as compared to glucosamine sulfate,[18] in a rat model of MIA induced OA.

2. Materials and methods

2.1. Animals

Thirty-two adult male Wister rats weighing 150–210 g at the start of the experiment were purchased from Moassat Animal House [Faculty of Medicine, Alexandria University]. Animals were fed standard rat chow with free access to water, and were acclimatized for 2 weeks before the experiment. The study protocol was approved by the Ethics Committee, Faculty of Medicine; Alexandria University, Egypt.

2.2. MIA-induced OA

OA was induced in rats [pre-anesthetized with ether] by a single intra-articular injection of 2 mg of mono-iodoacetate [MIA, Sigma-Aldrich, St. Louis, MO, USA] through the infrapatellar ligament into the joint space of the left knee, in a total volume of 25 μl saline, via a 26.5-G needle.[19] Control rats were injected with an equivalent volume of saline.

2.3. Animal grouping

Rats were assigned randomly into four groups, 8 rats in each group, as follows: Group I [control group], group II [MIA induced OA group], group III [MIA induced OA/glucosamine sulfate, 250 mg/kg/day, treated group],[20] and group IV [MIA induced OA/L-carnitine, 100 mg/kg, treated group].[21]

2.4. Knee diameter

Knee diameter was measured using calibrated digital caliper [World Precision Instruments, Stevenage, UK] in millimeter [mm] to assess the developmental stages of OA on days 0 [pre MIA injection], 1, 7, 14, 21, and 28 [post injection].[22]

2.5. Knee bend test

The Knee-bend test was done at days 0, 1, 7, 14, 21 and 28 to evaluate the movement-induced pain caused by MIA. Briefly, we recorded the squeaks and/or struggle reactions in response to five alternate flexion and extensions of the knee joint [performed within the physiological limits of knee flexion/extension] for each rat. The score of the test was determined as follows: 0 – no responses; 0.5 – struggle to maximal flexion/extension; 1 – struggle to moderate flexion/extension or vocalizations to maximal flexion/extension; 2 – vocalizations to moderate flexion/extensions. The sum of the animal's reactions, giving maximal values of 20, represents the Knee-Bend score, an indication of the animal's movement-induced nociception. The contralateral knee was always tested first, in order to avoid an increase in the contralateral score arising from the manipulation of the injected knee. Results for both ipsilateral and contralateral knees were presented.[23]

2.6. Biochemical analysis

On day 28, rats were anaesthetized with Thiopental sodium 40 mg/kg[24] intraperitoneally.[18] The left limb of the rat was flexed over a 20 ml glass vial, then 23-gauge needle was inserted, and the limb was secured in place with tape. Sterile saline was infused intra-articularly. 2 min after infusion of 100 μl of saline, the outflow fluid was aspirated. Synovial fluid was infused and withdrawn at a constant rate until a 400 μl basal sample was collected in a 1.5 ml centrifuge tube. Samples were immediately centrifuged, and the supernatants were

Please cite this article in press as: Ali SM et al. L-Carnitine ameliorates knee lesions in mono-iodoacetate induced osteoartL-carnitine for osteoarthritis in rats –>hritis in rats, *Alex J Med* (2016), http://dx.doi.org/10.1016/j.ajme.2016.03.002