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STRONTIUM RANELATE AS A NEW TREATMENT OF KNEE OSTEOARTHRITIS INDUCED BY MONOSODIUM IODOACETATE IN ALBINO RATS

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ABSTRACT

Objective: To evaluate the efficacy and the possible mechanism of action of strontium ranelate (SR) in the treatment of knee OA induced by monosodium iodoacetate (MIA) as a new indication of this drug, also, to detect whether cartilage oligomeric matrix protein (COMP) is a reliable biomarker that can facilitate the diagnosis of osteoarthritis (OA). **Methods:** twenty-four adult male *Swiss* albino rats weighing 150–200 g were assigned randomly into 4 groups, 6 rats in each: group I (control group), group II (SR-treated group), group III (MIA-induced OA) and group IV (MIA-induced OA + SR). Weights, knee diameters, knee bend scores were recorded on days 0, 1, 7, 14 and 28. On day 29 all animals were sacrificed, blood samples and synovial fluid of knee joints were collected; cartilage oligomeric matrix protein (COMP), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) levels were measured. The knee joints were removed and stained with H&E and Masson trichrome stains for histopathological and effect on collagen fibers evaluation. **Results:** Serum and synovial fluid levels of COMP, MDA, knee diameter and knee bend score were increased while SOD and CAT activities decreased in MIA-induced OA group associated with histopathological changes of OA. SR succeeded in amelioration of these parameters and preserve normal joint architecture compared to MIA-induced OA group. **Conclusions:** Higher serum and synovial COMP, however, reduction in antioxidant enzyme levels in MIA-induced OA. SR can ameliorate not only the antioxidant scavenging enzymes, but also pathological cartilage degradation, and can be considered as an effective drug in the management of knee OA.

KEYWORDS: Antioxidant enzymes, Cartilage oligomeric matrix protein, MDA, Osteoarthritis; Strontium ranelate, Monosodium iodoacetate.

INTRODUCTION

Osteoarthritis (OA) is an age-related condition and the leading cause of pain, disability and shortening of adult working life.[1] It is the most prevalent form of joint disease, affects as much as 13% of the world's population.[2] Knee OA is a disease of the "whole joint," which involves changes in the cartilage and subchondral bone.[3] It occurs due to an interaction between inflammatory, hypoxic and mechanical pathways.[4] The synovial tissue in the case of OA shows the production of free radicals and inflammatory mediators in the synovial fluid.[5]

The products of cartilage matrix degradation or turnover which appear in the blood, urine or synovial fluid have received much attention since they could serve as biomarkers for osteoarthritis.[6] One of the more promising markers is cartilage oligomeric matrix protein (COMP) which is located preferentially in the matrix of articular cartilage. Its function is not clear, but it may have a role in chondrogenesis and in the interaction of the chondrocyte with its surrounding matrix.[7,8]

There are no treatments marketed for structural disease modification, where current treatments mainly target symptoms.[4] Cardiovascular risks, renal disorder and gastrointestinal bleeding associated with non-steroidal anti-inflammatory drugs (NSAIDs) limit their long-term use.[4,9] Corticosteroids have serious adverse effects and intra-articular hyaluronans could cause damage at the site of action[10], hence, novel target receptors or pathways, which remain unaffected by conventional therapy, are being increasingly looked for.[11]

Strontium (Sr) is a trace element; which is recently licensed for the treatment of osteoporosis.[12,13] It is defined as a bone-seeking element because of its chemical and physical similarities to the calcium and about 98% of the total body Sr content is localized in bone tissues.[14,15] Strontium ranelate (SR) has a dual mode of action; simultaneously increasing bone formation while decreasing bone resorption.[16]

Due to the free radicals and inflammatory pathways in causing damage to the articular cartilage has become

generally accepted so, the rational of this study are to measure the COMP as circulating biomarker of matrix degradation, antioxidant enzymes as SOD and CAT, lipid peroxidation levels and signs of inflammation; knee diameter and knee bend score. The fact that OA is increasingly considered a disease in which periarticular bone changes take a central role was an additional motivation to evaluate the effectiveness of SR for the treatment of this disease.

MATERIALS AND METHODS ANIMALS

Twenty-four male adult *Swiss* albino rats weighing 150-200g have been used. Animals were obtained from the animal house Faculty of Medicine, Assiut University, Egypt and fed a standard diet of commercial rat chow and tap water. Rats were left to acclimatize to the environment for one week prior to inclusion in the experiment at Faculty of Medicine, Sohag University. Rats were maintained under standard laboratory conditions at an ambient temperature of 25±2°C, with 12-hour light/12-hour dark cycles. Animals were given a free access to food and water up to 24 hours prior to their use. This study was approved by the Institutional Animal Care and Use Ethical Committee of Faculty of Medicine, Sohag University.

Drugs and chemicals

SR was purchased from (Les Laboratoires Servier Industrie-France). Monosodium iodoacetate (MIA) was obtained from (Sigma-Aldrich Chemical Company, USA). ELISA kit for determination of COMP was obtained from Glory Science Co., Ltd USA. Kits for determination of oxidative stress parameters: (SOD, CAT, MDA) were obtained from the Biodiagnostic Company, Egypt.

Induction of osteoarthritis (OA)

OA was induced by a single intra-articular (i.a) injection of MIA into the right knee joint of rats after pre-anesthesia with ethyl ether. Each rat was positioned on their back and the right leg was flexed 90° at the knee. The patellar ligament was palpated and the injection was made into the infrapatellar ligament into the joint space. Each rat received 2 mg of MIA dissolved in 25µl physiologic saline (at a concentration of 80 mg/ml) i.a using a 27 gauge 0.5-inch needle syringe.[17,18]

Experimental design

Rats were randomly divided into four groups; six animals each, all animals received treatment via oral gavage. The first group was the control group received distilled water. The second group was treated with SR 500 mg/kg dissolved in distilled water.[19]

The third group; MIA-induced OA group and the fourth group is the treated MIA-induced OA group was administered SR 500 mg/kg one day after induction of OA. Animals received the treatment for 28 days. The first and second group were injected once i.a with an equivalent volume of saline in the right knee.

Body weight

Body weights of rats were measured weekly in grams (g) on days 0, 1, 7, 14, 21 and 28 (post injection).

Knee diameter

Right knee transverse diameter was measured weekly under light ethyl ether anesthesia using digital micrometer in millimeter (mm), which was used as an index for the degree of edema of the knee on days 0, 1, 7, 14, 21 and 28.[20]

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, animals were gently restrained, allowing access to both hind limbs while at the same time restricting movement.

The test consisted on counting the squeaks and/or struggle reactions in response to five alternate flexions and extensions of the knee joint, performed within the physiological limits of movement. The score of the test was determined by the type of reaction to each movement of the joint as follows: 0 - no responses to any kind of movement of the joint; 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. A maximal extension corresponds to placing the knee joint at 180° angle; a moderate extension corresponds to an angle between 120° and 150°, approximately; a moderate flexion corresponds to an angle between 45° and 75°, approximately; a maximal flexion corresponds to totally bending the knee joint (corresponding approximately to an angle of 30°). 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.[21]

Sample collection

On day 29, rats were anesthetized with thiopental sodium 40 mg/kg i.p. then, synovial fluid was aspirated. This was done by infusion of 100µl of 0.9% sterile saline into the synovial cavity of the right knee over a period of 2 minutes by 27 gauge needle. Two minutes after infusion of all the saline, fluid was withdrawn from the synovial cavity over a period of 2 minutes by a second outflow needle (27 gauge), which was inserted into the synovial cavity approximately 3 mm from the infusion needle.[22] The saline was infused and withdrawn at a constant rate until a 500µl sample was collected.[23] Then animals were sacrificed and blood samples were obtained.

Synovial aspirates and serum were separated by centrifugation at 3000 rpm for 10 minutes and divided into two sets of tubes; the first was kept at -20ºC for estimation of COMP and the second at -80 ºC for estimation of SOD, CAT and MDA levels.

Biochemical analysis Determination of COMP levels
The levels of COMP in the serum and synovial aspirate were measured using monoclonal double-antibody sandwich enzyme linked immunosorbent assay (ELISA) kits (Glory Science Co., Ltd USA) according to Yamanokuchi *et al.*[24] A standard solution was added to standard wells. 40 µl of sample were pipetted into the wells of a microtiter plate and then added both 10µl COMP-antibody labeled-biotin and 50µl Streptavidin-HRP. Then seal the membrane and gently shaking, incubated 60 minutes at 37°C. After washing, we added 50µl chromogen solution A, then 50µl chromogen solution B to each well. Gently mixed and incubated for 10 min at 37°C. Stop Solution was added 50µl into each well to stop the reaction and the reaction mixture was read spectrophotometrically using a Stat Fax 2600 Microplate Reader, USA) at 450 nm. The COMP levels in the samples were determined by comparing the optical density of the samples to the standard curve. The COMP levels were expressed in ng/ml.

Determination of SOD enzyme activity
SOD activity in serum and synovial fluid was determined by a colorimetric method which was described by Nishikimi *et al.*[25] This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye. The change in the absorbance was measured at 560 nm for control and sample at 25ºC. SOD activity was expressed in U/ml.

Determination of CAT activity
CAT activity in serum and synovial fluid aspirates was determined by a colorimetric method as described by Aebi.[26] This assay relies on the reaction of CAT with a known quantity of H2O2. The reaction is stopped after exactly one minute with CAT inhibitor. In the presence of peroxidase, the remaining H2O2 reacts with 3, 5-Dichloro-2-hydroxy- benzene sulfonic acid (DHBS) and 4-Amino-phenazone (AAP) to form a chromophore. The change in the absorbance was measured at 510 nm. CAT activity was expressed in U/L.

Determination of lipid peroxidation levels
Measurement of serum and synovial fluid MDA levels as an indicator to lipid peroxidation was done by a colorimetric method which described by **Ohkawa *et al.***[27] The principle of the method is based on spectrophotometric measurement of the color formed during the reaction of the thiobarbituric acid with MDA. They react in acidic medium at a temperature of 95ºC for 30 min to form a thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534nm. MDA level was expressed in nmol/ml.

Tissue preparation (for light microscopic study)
The right knee joint was dissected and the soft tissues around it were removed. The knee joints were fixed in 10% paraformaldehyde for 48 h. The joints were decalcified in 10% EDTA for 3 weeks and embedded in a paraffin block. The samples were cut into 5 µm sections and were stained with hematoxylin and eosin for general histological study and with Masson's trichrome stain for demonstration of collagen fibers.[28]

Statistical analysis
The results were presented as mean values ±S.E.M. The SPSS version 17.0 was used in statistical analysis. Biochemical markers among different groups were compared using the one-way ANOVA test. When a significant difference was observed, post hoc Tukey test analysis was carried on for multiple comparisons. Post hoc Bonferroni correction was used in repeated measures. A difference was considered significant when the $p < 0.05$.

RESULTS WEIGHT
(Fig. 1) revealed that initial body weights (b.w.) were similar among all groups at day 1 and 7 compared to control group. However, a significant increase ($p<0.05$) in b.w. started to occur at day14; in SR and MIA+SR groups and the increment in b.w. were maintained until the end of the study. On the other hand, at day 14, 21 and 28 there was a significant reduction ($p<0.05$) in b.w.in MIA group compared to the corresponding time in the control group.

Knee diameter
There were no significant changes in the right knee diameters between groups at the first day after i.a. administration of the saline or MIA compared to control group. MIA administration i.a produced significant increase ($p<0.001$) in the diameter and the maximal effect started at the day 7 and 14 compared to the control in the same group. Treatment of rats with OA by SR produced decrease in right knee diameter started after14 day of treatment and became significant ($p< 0.05$) after 21 and 28 days of treatment as compared to the corresponding time in MIA group (Fig. 2).

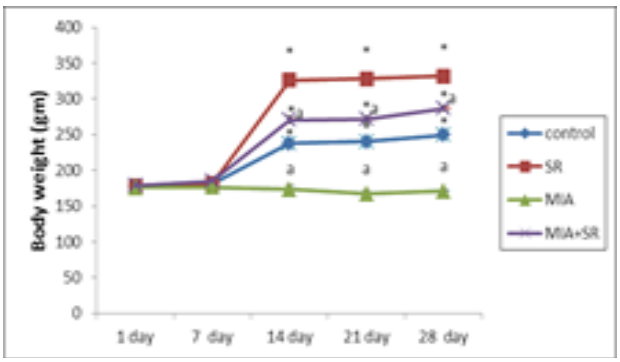

Figure 1: Effect of oral SR (500 mg/kg/day) on the body weight (gm) in the rat model of MIA-induced osteoarthritis. Each value represents the mean ± SEM. N= 6. SR=strontium ranelate. MIA= monosodium iodoacetate. a Significant at ($p<0.05$) vs. control group at the corresponding time. * Significant at ($p<0.05$) vs. body weight at day 1 in the same group.

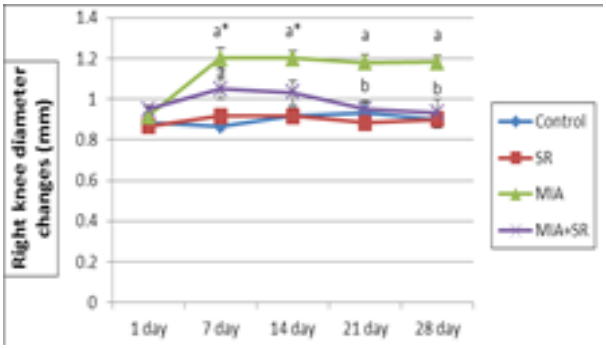

Figure 2: Effect of oral SR (500 mg/kg/day) on the right knee diameters in the rat model of MIA-induced osteoarthritis. Each value represents the mean ±SEM. N= 6. SR=strontium ranelate. MIA= monosodium-iodoacetate. a Significant at (p<0.05) vs. control group at the corresponding time. b Significant at (p<0.05) vs. MIA-treated group at the corresponding time.
*** Significance at (p<0.05) vs. day 1 in the same group.**

Knee bends score

No sign of spontaneous nociceptive behavior or distress was found in control or SR groups where there were no significant differences between these groups at different time intervals. In MIA group there was a significant increase (p< 0.05) in knee bend score started at 1st day and the peak values occurred at 7th and 14th days compared to the control group. Treatment of OA with SR in the fourth group resulted in significant decrease (p< 0.05) in knee bend score started at 7th day compared to MIA group and the maximal response occurred at 28th day (Table 1).

Table 1: Effect of oral SR (500 mg/kg/day) on the right Knee Bend score in the rat model of MIA-induced osteoarthritis.

	Groups	Days				
		1	7	14	21	28
Right Knee Bend Score	Control	2.92±0.23	3.58±0.24	2.83±0.17	2.67±0.11	2.75±0.21
	SR	2.58±0.23	3.00±0.13	2.58±0.15	2.50±0.13	2.42±0.15
	MIA	5.25 a±0.21	11.92 *a±0.15	11.42 *a±0.15	9.42 *a±0.24	9.25 *a±0.34
	MIA+SR	5.00 a±0.18	5.33 ab±0.17	4.67 ab±0.17	3.92 *ab±0.20	3.58*b±0.15

Each value represents the mean ±SEM. N= 6. SR=strontium ranelate. MIA= monosodium-iodoacetate. [a]Significant at (p<0.05) vs. control group at the corresponding time.
*[b]Significant at (p<0.05) vs. MIA-treated group at the corresponding time. * Significance at (p<0.05) vs. day 1 in the same group.*

Biochemical markers

Serum and synovial COMP levels

Single i.a. injection of rats with 2 mg of MIA produced a significant increase (p<0.01) in serum and synovial COMP level compared to control group, where, treatment of OA with 500 mg/kg b.w. for 28 days duration produced a significant decrease (p<0.01) in serum and synovial COMP level compared to MIA group (Fig. 3 and 4).

Serum and synovial SOD activities

Administration of MIA as a single i.a. injection to the rats induced a significant decrease (p<0.01) in serum and synovial activities of SOD compared to control group. However, treatment of rats with OA by SR in a dose of 500 mg/kg/day for 28 days led to a significant increase (p<0.01) in SOD in the serum and synovial fluid compared to MIA group as shown in the table (2 and 3).

Serum and synovial CAT activities

As shown in the table (2 and 3), administration of MIA once i.a to the rats induced a significant reduction (p<0.01) in the serum and synovial activities of CAT compared to control group. On the other hand, administration of SR in a dose of 500 mg/kg b.w. daily for 28 days to osteoarthritic rats led to a significant elevation in CAT activity compared to MIA group and the SR could return the CAT activity near to the normal where there was no significant difference between MIA+SR group and control group in cases of serum and synovial fluid (p=0.159 and p=0.168) respectively.

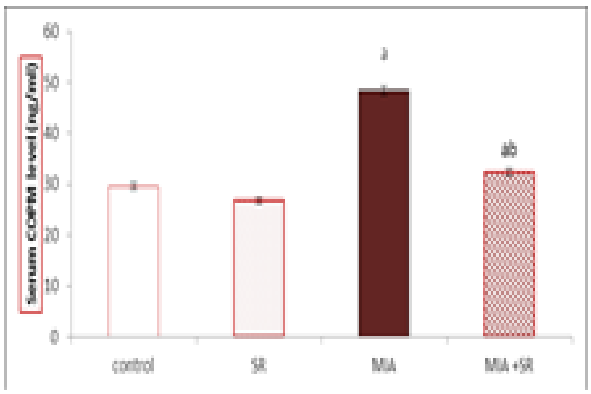

Figure 3: Effect of oral SR (500 mg/kg/day) on serum levels of COMP in the rat model of MIA-induced osteoarthritis. Each value represents the mean ±SEM. N=6. SR=strontium ranelate. MIA= monosodium-iodoacetate. COMP= cartilage oligomeric matrix protein. [a] Significant at (p<0.05) vs. control group. [b] Significant at (p<0.05) vs. MIA-treated group.

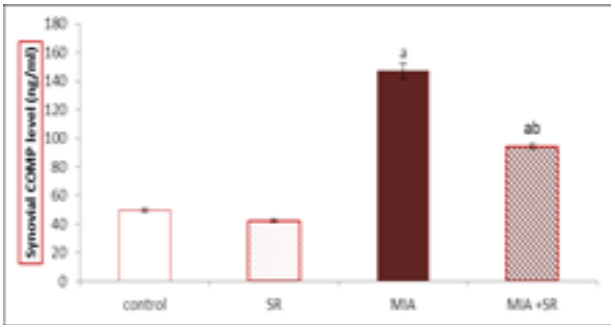

Figure 4: Effect of oral SR in (500 mg/kg/day) on synovial levels of COMP in the rat model of MIA-induced osteoarthritis. Each value represents the mean ±SEM. N=6. SR=strontium ranelate. MIA= monosodium-iodoacetate. COMP= cartilage oligomeric matrix protein.
[a] Significant at (p<0.05) vs. control group.
[b] Significant at (p<0.05) vs. MIA-treated group.

Serum and synovial MDA levels

Single i.a. injection of 2 mg of MIA to the rats produced significant increase (p<0.01) in serum and synovial MDA level compared to control groups, however, treatment of osteoarthritic rats with SR in a dose of 500 mg/kg b.w. for 28 days duration, produced significant decrease (p<0.01) in serum and synovial MDA level compared to MIA groups and SR succeeded in return the serum MDA level (not the synovial) near to the control value where there was no significant difference with corresponding control group (p= 0.145), (Table 2 and 3).

Table 2: Effect of oral SR in (500 mg/kg/day) on serum levels of SOD, CAT and MDA in the rat model of MIA-induced osteoarthritis.

	Groups	Control	SR	MIA	SR+MIA
Serum	SOD (U/ml)	0.97 ± 0.08	1.05± 0.04	0.63 a± 0.03	0.95 b± 0.06
	CAT (U/L)	1.60 ± 0.05	1.65± 0.02	1.08 a± 0.06	1.47 b± 0.04
	MDA (nmol/ml)	3.50 ±0.18	3.23± 0.09	6.65 a± 0.15	4.03 b± 0.22

Each value represents the mean ±SEM. N= 6. SR=strontium ranelate. MIA= monosodium-iodoacetate. SOD=superoxide dismutase. CAT=catalase. MDA= malondialdehyde. [a] Significant at (p<0.05) vs. control group. [b] Significant at (p<0.05) vs. MIA-treated group.

Synovial

Table 3: Effect of oral SR (500 mg/kg/day) on synovial fluid aspirate levels of SOD, CAT and MDA in the rat model of MIA-induced osteoarthritis.

	Groups	Control	SR	MIA	SR+MIA
Synovial	SOD(U/ml)	1.95 ± 0.04	2.18 ± 0.08	0.67 a ± 0.04	1.05 ab ± 0.06
	CAT (U/L)	1.82 ± 0.04	1.77 ± 0.07	1.37 a ± 0.02	1.97 b ± 0.06
	MDA (nmol/ml)	4.97 ± 0.10	4.48 ± 0.11	7.80 a ± 0.08	5.85 ab ± 0.06

Each value represents the mean ±SEM. N= 6. SR=strontium ranelate. MIA= monosodium-iodoacetate. SOD=superoxide dismutase. CAT= catalase. MDA= malondialdehyde. [a]Significant at (p<0.05) vs. control group. [b]Significant at (p<0.05) vs. MIA-treated group.

Histological Analysis of the Knee Joint Light Microscopic Results

Joint histology was assessed in all animals. Control and SR groups showed a normal joint space, regular hyaline cartilage and surface with normal homogenous and diffuse collagen fibers (Fig. 5 a & b and 6 a & b). In contrast, MIA administration led to loss of articular cartilage integrity, destructive, necrotic changes of the chondrocytes and absence of the cartilage with pyknosis and karyolysis in addition, decrease the collagen fiber content (Fig 5 c, d & e and 6 c). Histological signs of cartilage degradation were reversed in MIA-induced OA rat treated with SR for 28 days duration with normal joint space, articular cartilage surface and collagen fiber content (Fig 5 f & 6 d).

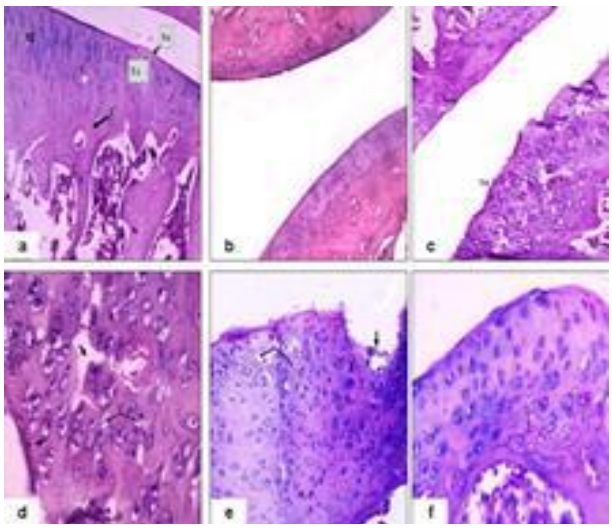

Figure 5: A photomicrograph of a section in the knee joint of (a) Control adult rat showing the tibia with

normal regular basophilic articular cartilage without covering perichondrium. It displays superficial zone (SZ), radial zone (RZ) and transitional zone (TZ) with intact tide mark separating it from calcified cartilage (C) and subchondral bone (arrow). Note vertical linear organization of chondrocytes in the radial zone. (b) SR treated group showing normal joint structure. (c) MIA-induced OA showing; normal femoral aspect but degenerative changes in the tibial aspect. (d) Magnified view of the tibia of MIA-induced OA showing loss of articular cartilage integrity, destructive and complete absence of cartilage (arrow) in addition to pyknosis (P), karyolysis (K) and vaculation (V) of the chondrocytes. Note absence of the tide mark. (e) Magnified view of the tibia of MIA-induced OA exhibits surface irregularity (fraying), localized area of erosion of the articular surface in association with vacuolation or rarefaction of the matrix (v) and disorganization of the chondrocyte with absent of the tide mark. (f): Tibia of a **rat with MIA-induced OA treated with SR for 28 days duration adult rat showing smooth articular cartilage surface, healthy chondrocytes normal calcified cartilage (arrow) (a,b,c) H&E X 200, (d,e,f); H&E X 400.**

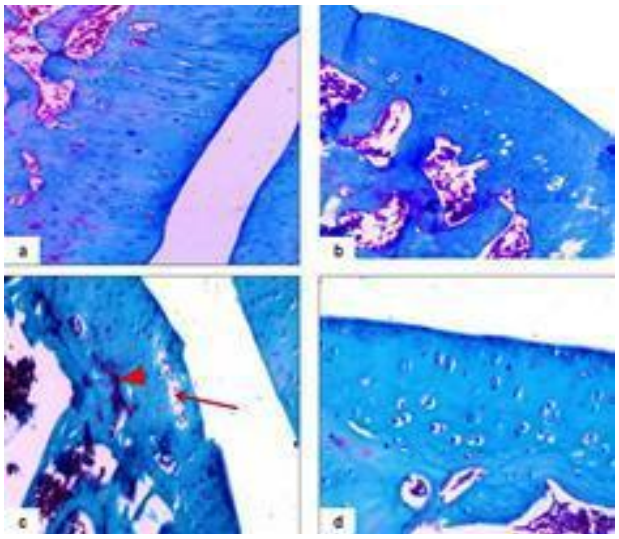

Figure 6: A photomicrograph of a section of knee joint of (a) Control rat show prominent, homogenous and diffuse bluish staining of the collagen fibers in the different zones. (b) SR treated group showing normal homogenous and bluish staining of the collagen fibers. (c) MIA-induced OA showing; decreased collagen fiber content which presented by faint staining, complete loss of blue color in some areas (arrow) and heterogonous staining in others (arrow head). (d) MIA-induced OA rat treated with SR for 28 days duration showing normal homogenous and bluish staining of the collagen fibers. Masson trichrome stain X 200.

DISCUSSION

Osteoarthritis (OA) isa degenerative joint disease characterized by a progressive loss of cartilage, changes in subchondral bone and chronic pain.[3] The search for drugs which able to slow the progression of the disease is a great challenge and would meet an important medical need for patients with OA. The development and progression of OA are now believed to involve inflammation, oxidative stressand cartilage and subchondral bone changes.[4]

The MIA-induced OA animal model used in the present study is a well-characterized model and mimics pain and biochemical/structural changes associated with human OA.[29, 30] Its mechanism is via the inhibition of the activity of glyceraldehyde-3-phosphate dehydrogenase in chondrocytes, resulting in disruption of glycolysis with a loss of proteoglycan matrix and eventually cell death.[29, 30, 31]

COMP is one of the essential components of the extracellular matrix of the cartilage and is considered as a marker of the cartilage breakdown. It regulates the cellular proliferation, apoptosis and cellular attachment in the cartilaginous tissue.[32]

In our study, MIA-induced OA which proved by an increase in knee diameter, knee bend score and by the microscopic picture of the knee joint, associated with an elevation in the level of COMP in both serum and synovial fluid. This effect is presumably due to high turnover rate by the chondrocytes in order to repair the cartilage matrix and the degradation products of cartilage metabolism are released into the synovial fluid and thereafter into the blood.[33]

Our results are in agreement with many studies done on human and animal OA model.[8, 34, 35, 36, 37] In contrast to our result, Misumi *et al*.[38] reported that the serum concentration of COMP in OA horses was significantly reduced compared to the control. The discrepancy between previous study and our result may depend on the stage of OA; suggested that serum COMP values increase early in the course of OA and then decrease at the later stage[39, 40] and the different affinity of primary antibodies used to detect COMP.[41] On the other hand, our results showed that SR played a role in the reversal of OA induced by MIA, confirmed by the histopathology of the joints and the reduction in the marker of the cartilage degradation levels in serum and synovial fluid with a reduction in the knee diameter and knee bend score. This result is in harmony with that done by Liu *et al*.[42] where they found that cartilage degradation and subchondral bone loss were reversed by treatment of SR in mandibular OA in rats. The explanation of these findings are supported by that, SR could largely attenuate cartilage degeneration and then decrease COMP levels by inhibition of TGF-β signaling, where this pathway leads to OA-like pathological changes.[43,44] SR, also significantly attenuates cartilage matrix and chondrocyte loss by improving the expression of SOX9; a critical transcription factor responsible for the expression of anabolic genes type II collagen and aggrecan.[45] In

addition, Chen *et al*.[46] suggested that SR has anti-inflammatory effect where it penetrates into the cartilage tissue and acts directly on chondrocytes inhibiting the NF-Kb (nuclear factor-kappa B) signal which have multiple effects on chondrocytes metabolism alleviating the cartilage degradation and synovial inflammation in the joint. Knudsen *et al.*[47] and Buache et al.[48] suggested the anti-inflammatory effect of SR is through the inhibition of Na (+) -K+ pump in mast cell plasma membrane leading to its stabilization and prevention of release of its inflammatory mediators and also due to inhibition of production of TNF-α and IL-6, powerful activators of immune cells, reducing the production of the proteolytic mediators, which cause tissue injuries and aseptic loosening.

Antioxidant enzymes e.g., SOD, CAT, are the primary defense against oxidative damage of tissues. SOD defense against oxygen-derived free radicals, converting the superoxide anion (O2•-) into H2O2. CAT is the main enzyme involved in removing H2 O2, which is generated from superoxide anion radicals by SOD.[19] MDA, a product of lipid peroxidation can not only influence the degree of cell damage but also reflects the severity of lipid peroxidation. The elevation in MDA inhibits the cartilage cell proliferation by inhibition of DNA synthesis thus accelerating cartilage matrix degradation and cell death.[49]

In our study, there were a reduction in SOD and CAT enzyme activities in OA rats and elevation in MDA in the serum and synovial fluid; where SR succeeded to ameliorate these parameters near to the control levels. Our results are in agreement with studies done by Yalin *et al.*[19] & El-Megharbel *et al.*[50] which proved that SR treatment led to amelioration in SOD, CAT and MDA in osteoblast and endothelial cell *in vitro* and *in vivo* respectively. In addition, Maneesh *et al.*[51] & Li *et al.*[52] reported that the reduction in serum and erythrocytes SOD, CAT, glutathione reductase and elevation in MDA levels in rat knee OA was correlated with OA severity.

The SR can reduce ROS (reactive oxygen species) in tissues by stimulation of ATP provided by the mitochondria, on the other hand, it is effective in increasing the antioxidant enzyme activity which eliminates ROS.[53] Interestingly, SR suppresses ROS by regulating the P450scc which is closely related to ROS production.[52] Preclinical studies have shown that SR enhances preosteoblast replication and promotes osteoblastic differentiation and inhibits osteoclastic activity leading to prevention of the resorption of subchondral bone.[16,54,55] This effect may be linked to activation of calcium-sensing receptors in bone cells.[56,57] These positive effects on bone made SR an interesting candidate for the treatment of OA. In addition, SR stimulated the production of insulin-like growth factor 1 (IGF-1) and boosted the stimulating effect of IGF-1 on proteoglycan synthesis in chondrocytes in a human model.[58,59,60] SR may also inhibit the resorption of subchondral bone by modulating matrix metalloproteinases (MMP-2 and MMP-9), which degrade type I collagen, fibronectin and aggrecans.[16]

CONCLUSION

This study demonstrated that MIA induced OA with oxidative stress and altered marker of cartilage degradation associated with abnormal joint histopathology.

COMP has shown promise as a potential biomarker for monitoring progression of cartilage destruction, for evaluating the effect of therapy and as a prognostic tool reflecting cartilage damage. Proposed a promising use of SR as a potential therapy of OA where it not only by relieves its symptoms but also through its action on the underlining pathology including its cartilage preservation, antioxidant and anti-inflammatory effects.

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REFERENCES

1. Marker C and Pomonis J. The monosodium iodoacetate model of osteoarthritis pain in the rat. Methods Mol Biol, 2012; 851: 239-248.
2. Tonge D, Pearson M, Jones y. The hallmarks of osteoarthritis and the potential to develop personalised disease-modifying pharmacological therapeutics. Osteoarthritis Cartilage, 2014; 22(5): 609-621.
3. Abramson S and Attur M. Developments in the scientific understanding of osteoarthritis. Arthritis Res Ther, 2009; 11(3): 227.
4. Keny M, Sushama A, Padmanabh V, Rataboli S. Knee osteoarthritis -a pathological basis for use of newer drug therapies. Int J Basic Clin Pharmacol, 2014; 3(3): 424-430.
5. Benito M, Veale D, FitzGerald O, Bresnihan B. Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis, 2005; 64: 1263-1267.
6. Steffey M, Miura N, Rory J. Todhunter B. The potential and limitations of cartilage-specific (VDC) K fibronectin and cartilage oligomeric matrix protein as osteoarthritis biomarkers in canine synovial fluid. Osteoarthritis Cartilage, 2004; 12: 818-825.
7. Allen R and Kirby K. Nocturnal leg cramps. Am Fam Physician, 2012; 15; 86(4): 350-355.
8. Hawke F, Chuter V, Burns A. Impact of nocturnal calf cramping on quality of sleep and health-related quality of life. J Qual Life Res, 2013; 22(6): 1281-1286.
9. Hinton R, Moody R, Davis A, Thomas S. Osteoarthritis: diagnosis and therapeutic considerations. Am Fam Physician, 2002; 65: 841–848.

10. Bellamy N, Campbell J, Robinson V, Gee T. Intraarticular corticosteroid for treatment of osteoarthritis of the knee. 2005; Cochrane Database Syst Rev, (2): CD005328.
11. Barron M and Rubin B. Managing osteoarthritic knee pain. J Am Osteopath Assoc, 2007; 107: 21–27.
12. Roux C, Reginster J, Fechtenbaum J, Kolta S. Vertebral fracture risk reduction with strontium ranelate in women with postmenopausal osteoporosis is independent of baseline risk factors. J Bone Miner Res, 2006; 21: 536–542.
13. Boyd S Szabo E, Ammann P. Increased bone strength is associated with improved bone microarchitecture in intact female rats treated with strontium ranelate: A finite element analysis study. Bone, 2011; 48: 1109–1116.
14. Effah Kaufmann, E, Ducheyne P, Shapiro I. Evaluation of Osteoblast Response to Porous Bioactive Glass (45S5) Substrates by RT-PCR Analysis. Tissue Eng, 2004; 6(1): 19-28.
15. Fernandez-Garcia D, Alonso G, Munoz-Torres M. Anabolic therapy of osteoporosis. Med Clin, 2005; 125: 341-345.
16. Tat S, Pelletier J, Mineau F, Caron J. Strontium ranelate inhibits key factors affecting bone remodelling in human osteoarthritic subchondral bone osteoblasts. Bone, 2011; 49: 559–567.
17. Guzman R, Evans M, Bove S, Morenko B. Monoiodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. Toxicol Pathol, 2003; 31(6): 619–624.
18. Combe R, Bramwell S, Field M. The monosodium iodoacetate model of osteoarthritis: a model of chronic nociceptive pain in rats? Neurosci Lett, 2004; 11; 370(2-3): 236-240.
19. Yalin S, Sagir O, Comelekoglu U, Berköz M. Strontium ranelate treatment improves oxidative damage in osteoporotic rat model. Pharmacol Rep. 2012; 64(2): 396–402.
20. Khan M, Ashraf M, Hashmi A, Ahmad MD, Anjum AA. Clinical assessment of experimentally induced osteoarthritis rat model in relation to time. Anim Plant Sci, 2012; 22(4): 960–965.
21. Ferreira-Gomes J, Adães S and Castro-Lopes J. Assessment of Movement-Evoked Pain in Osteoarthritis by the Knee-Bend and CatWalk Tests: A Clinically Relevant Study. J Pain, 2008; 9(10): 945-954.
22. Levy A, Simon O, Shelly J, Gardener M. 6-shogaol reduced chronic inflammatory response in the knees of rats treated with complete Freund's adjuvant. BMC Pharmacol, 2006; (1)6: 12.
23. Cialdai C, Giuliani S, Valenti C, Tramontana M. Effect of Intra-articular 4-(*S*)- Amino- 5-(4-{4-[2,4-dichloro-3-(2,4-dimethyl- 8-quinolyloxym- ethyl) phenylsulfonamido]- tetrahydro-2*H*- 4-pyranylcarbonyl} piperazino)- 5-oxopentyl] (trimethyl)ammonium chloride hydrochloride (MEN16132), a Kinin B2 Receptor Antagonist, on Nociceptive Response in Monosodium Iodoacetate-Induced Experimental Osteoarthritis in Rats. J Pharmacol Exp Ther, 2009; 331(3): 1025-1032.
24. Yamanokuchi K, Tagami M, Nishimatsu E, Shimizu Y. Sandwich ELISA system for cartilage oligomeric matrix protein in equine synovial fluid and serum. Equine Vet J, 2009; 41(1): 41-46.
25. Nishikimi M, Roa A, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun, 1972; 64(2): 849-854.
26. Aebi H. Catalase in vitro. Methods Enzymol, 1984; 105: 121-126.
27. Ohkawa, K., Ohishi, W., Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 1979; 95(2): 351-358.
28. Gamble M, Wilson I. Hematoxylin and eosin. In: Bancorft D & Gamble M (eds.). Theory and practice of histological techniques, 5th edition, Toronto, London, New York; Churchil Living Stone. 2002; 125-146.
29. Bendele A. Animal models of osteoarthritis. J Musculoskel Neuron Interact, 2001; 1(4): 363-376.
30. Sulzbacher I. Osteoarthritis: histology and pathogenesis. Wien Med Wochenschr, 2013; 163(9–10): 212–219.
31. Beyreuther B, Callizot N, Stohr T. Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain. Arthritis Res Ther, 2007; 9(1): 14.
32. Das B, Roy A, Khan F. Cartilage oligomeric matrix protein in monitoring and prognostication of osteoarthritis and its utility in drug development. Prespect Clin Res, 2015; 6(1): 4-9.
33. Verma P, Dalal K. Serum cartilage oligomeric matrix protein (COMP) in knee osteoarthritis: a novel diagnostic and prognostic biomarker. J Orthop Res, 2013; Jul; 31(7): 999-1006.
34. Vilím V, Olejárová M, Machácek S, Gatterová J Serum levels of cartilage oligomeric matrix protein (COMP) correlate with radiographic progression of knee osteoarthritis. Osteoarthritis Cartilage, 2002; 10(9): 707-713.
35. Senolt, leja, orejtova, atterova. Increased pentosidine, an advanced glycation end product, in serum and synovial fluid from patients with knee osteoarthritis and its relation with cartilage oligomeric matrix protein. Ann Rheum Dis, 2005; 64: 886–890.
36. Kong S, Stabler T, Criscione L, Elliott A. Diurnal Variation of Serum and Urine Biomarkers in Patients With Radiographic Knee Osteoarthritis. Arthritis Rheum, 2006; 54(8): 2496–2504.
37. Zivanović, Rackov L, Zivanović A, evtić. Cartilage oligomeric matrix protein - inflammation biomarker in knee osteoarthritis. Bosn J Basic Med Sci, 2011; 11(1): 27-32.

38. Misumi M, Vilim V, Clegg P. Measurement of cartilage oligomeric matrix protein (COMP) in normal and diseased equine synovial fluids. Osteoarthritis Cartilage, 2001; 9: 119–127.
39. Forslind K, Eberhardt K, Jonsson A, and Saxne T. Increased serum concentrations of cartilage oligomeric matrix protein. A prognostic marker in early rheumatoid arthritis. Br J Rheumatol, 1992; 31(9): 593-598.
40. Sharif M, George E, Dieppe P. Correlation between synovial fluid markers of cartilage and bone turnover and scintigraphic scan abnormalities in osteoarthritis of the knee. Arthritis Rheum, 1995; 38(1): 78-81.
41. Hummel K, Neidhart M, Vilim V, Hauser N. Analysis of cartilage oligomeric matrix protein (COMP) in synovial fibroblasts and synovial fluids. Br J Rheumatol, 1998; 37(7): 721-728.
42. Liu Y, Yang H, Liao L, Jiao K, Zhang H, Lu L. Systemic administration of strontium or NBD peptide ameliorates early stage cartilage degradation of mousemandibular condyles. Osteoarthritis Cartilage, 2016; 24(1): 178-87.
43. Zhen G, Wen C, Jia X, Li Y, Crane J. Inhibition of TGF-beta signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. Nat Med, 2013; 19: 704–712.
44. Jiao K, Zhang M, Niu L, Yu S, Zhen G, Xian L. Overexpressed TGF-beta in subchondral bone leads to mandibular condyle degradation. J Dent Res, 2014; 93: 140–147.
45. Yu D, Ding H, Mao Y, Liu M, Yu B, Zhao X. Strontium ranelate reduces cartilage degeneration and subchondral bone remodeling in rat osteoarthritis model. Acta Pharmacol Sin, 2013; 34: 393–402.
46. Chen L, Lin L, Wang H, Wei X, Fu X, Zhang J. Suppression of early experimental osteoarthritis by in vivo delivery of the adenoviral vector-mediated NF-kappaBp65-specific siRNA. Osteoarthritis Cartilage, 2008; 16: 174–184.
47. Knudsen T. The Na+/K(+)-pump in rat peritoneal mast cells: some aspects of regulation of activity and cellular function. Dan Med Bull, 1995; Nov; 42(5): 441-54.
48. Buache E, Velard F, Bauden E, Guillaume C. Effect of strontium-substituted biphasic calcium phosphate on inflammatory mediators production by human monocytes. Acta Biomaterialia, 2012; 8: 3113–3119.
49. Loeser R, Carlson C, Del Carlo M, Cole A. Detection of nitrotyrosine in aging and osteoarthritic cartilage: correlation of oxidative damage with the presence of interleukin-1β and with chondrocyte resistance to insulin-like growth factor 1. Arthritis Rheum, 2002; 46: 2349–2357.
50. El-Megharbel S, Hamza R, Refat M. Synthesis, spectroscopic and thermal studies of Mg(II), Ca(II), Sr(II) and Ba(II) diclofenac sodium complexes as anti-inflammatory drug and their protective effects on renal functions impairment and oxidative stress. Spectrochim Acta A Mol Biomol Spectrosc, 2015; 25; 135: 915-928.
51. Maneesh M, Jayalekshmi H, Suma T, Chatterjee S, Chakrabarti A. Evidence for oxidative stress in osteoarthritis. Indian J Clin Biochem, 2005; 20(1): 129-130.
52. Li J, Hu Y, Zhang Q, Ma B, Wu Z, Wang Y. Strontium fructose 1, 6-diphosphate alleviates cyclophosphamide-induced oligozoospermia by improving antioxidant and inhibiting testicular apoptosis via FAS/FASL pathway. Andrologia, 2015 Nov; 47(9): 995-1003.
53. Vaamonde-Garcia C, Riveiro-Naveira R, Valcárcel-Ares M. Mitocho Marie P. (2007): Strontium ranelate: New insights into its dual mode of action. Bone, 2012; 40: S5-S8.
54. Henrotin Y, Labasse A, Zheng S, Galais P. Strontium ranelate increases cartilage matrix formation. J Bone Miner Res, 2001; 16: 299–308.
55. Marie P. Strontium ranelate: New insights into its dual mode of action. Bone, 2007; 40: S5-S8.
56. Chattopadhyay N, Quinn S, Kifor O, Ye C, Brown E. The calcium-sensing receptor (CaR) is involved in strontium ranelate-induced osteoblast proliferation. Biochem Pharmacol, 2007; 74: 438–447.
57. Brennan T, Rybchyn M, Green W, Atwa S. Osteoblasts play key roles in the mechanisms of action of strontium ranelate. Br J Pharmacol, 2009; 157: 1291–1300.
58. Gulhan I, Bilgili S, Gunaydin R, Gulhan S, Posaci C. The effect of strontium ranelate on serum insulin like growth factor-1 and leptin levels in osteoporotic post-menopausal women: a prospective study. Arch Gynecol Obstet, 2008; 278: 437–441.
59. Narmazi H, Cooper C, Reginster J. Efficacy and safety of oral strontium ranelate for the treatment of knee osteoarthritis: rationale and design of randomised, double-blind, placebo-controlled trial. Curr Med Res Opin, 2012; 28: 609–610.
60. Reginster J, Pelousse F, Bruyère O. Is there potential for strontium ranelate in the management of osteoarthritis? Clin Pract, 2013; 10: 201–207.