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Nicotinate Supplements Slow Onset and Severity of Symptoms in the Monosodium Iodoacetate Rat Model for Osteoarthritis

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Keywords: Osteoarthritis; Monosodium iodoacetate rat model; Niacin; Niacinamide; Dynamic weight bearing; Joint diameter; Histophathology.

Abstract

Objective: Osteoarthritis (OA) is a major, heterogenous condition with main symptoms of pain and inflammation in the affected joints. In this study, we aim to establish a Monosodium Iodoacetate (MIA) rat model appropriate to assess the effects of supplements on the onset of the mild to moderate osteoarthritis.

Design: Different doses of MIA (0.5, 1 and 2 mg) were injected into rats to induce OA-like symptoms. Dynamic Weight Bearing (DWB) was used to assess pain, and joint diameter measures were used to assess inflammation in groups of rats (10 animals per group), either naïve rats with no injections or rats that had received a single intra-articular injection with MIA. Changes in DBW and joint diameter were recorded at day 3, 7, 14 and 24 after injection. In addition, effects of 400 mg/kg niacinamide or 80 mg/kg niacin on the MIA-OA induced symptoms were assessed. Histopathological examinations of knee joints were performed.

Results: A dose-dependent effect on DWB (weight deficit in right hind paw) caused by single injections of MIA could be observed in all MIA groups. These effects were not ameliorated by either niacinamide or niacin. However, a reduction in joint circumference was observed with niacinamide supplementation which reached statistical significance at day 7. The niacin groups also showed some alleviating effects on joint swelling, although the results did not reach statistical significance. Histopathological examinations showed dose-dependant MIA-induced microscopic changes. Niacinamide at 400 mg/kg showed an improvement in histological parameters with the 0.5 mg MIA dose, supporting the effects observed in reducing joint diameter. No effects were seen with Niacin.

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Conclusion: These results suggest that rats injected with a 0.5 mg dose of MIA are a suitable model to study the effects of nutritional supplements on mild to moderate onset of OA. In addition, the improvement of joint diameter reflecting an improvement of inflammation observed with niacinamide at 400 mg/kg and MIA at 0.5 mg suggest that niacinamide can be used to mitigate inflammation in mild osteoarthritis conditions.

Introduction

The water-soluble B_3 vitamins consisting of niacin (nicotinic acid) and niacinamide (nicotinamide, theamide of nicotinic acid), plays an essential role in energy generation by supplying NAD and NADP [1]. These nicotinates have also been shown to lower cholesterol [2], modulate the immune system [3], and to have an anti-inflammatory role [4]. As an essential vitamin, vitamin B_3 has a Recommended Daily Allowance (RDA) of 16 mg/day (males), 14 mg/day (females), 18 mg/day (pregnant females), 17 mg/day (breastfeeding females), and 20 mg/day (adults 60-90 years old) in the USA [5]. These levels vary in different regions. In the European Union, the Nutrient Reference Value (NRV) for niacin is 16 mg [6], while the European Food Safety Authority (EFSA) defines an average requirement (AR) for niacin of 1.3 mg Niacin Equivalents (NE)/MJ (corresponds to 5.5 mg NE/1000 kcal) [7].

Vitamin B_3 is usually taken up from food. Foods naturally rich in this vitamin include meat, legumes, nuts, coffee, and tea. Vitamin B_3 deficiency has been reported to result in pellagra, a condition of joint deterioration and skin desquamation which can lead to death if untreated [8], suggesting a link between vitamin B_3 deficiency and arthritis.

Osteoarthritis (OA) is one of the major and most impactful diseases in developed and developing countries and the most common form of arthritis, affecting 3.3 % of the world population [9] and OA of the knee was found to be 28.7% of a representative sample in India [10]. It presents as deterioration and subsequent loss of articular cartilage [11].

Nicotinates have been used successfully in the treatment of Rheumatoid Arthritis (RA), a more severe joint disease, since 1941 [12]. Dr. William Kaufman describes the outcome of 455 patients receiving 1,500-4,000 mg/d of niacinamide for periods of 1-2 months to reverse RA symptoms and improve joint mobility [13].

Confirming these results, Hoffer describes six cases where daily dosages of nicotinic acid or nicotinamide ranging from 1 to 3 grams per day over periods ranging from 1 month to several years improved symptoms of osteoarthritis, rheumatoid arthritis, schizophrenia, arthritis, and vascular nodulitis in subjects of both genders with ages from 14 to 68. Improved symptoms were impaired joint mobility, joint deformation, swelling, and inflammation. In all six cases, the treatment produced remissions of these forms of arthritis to a normal or nearly normal health state with the first effects becoming noticeable after 7-10 days. For two cases, a lessening of joint paint was also described [12].

A double-blind, placebo-controlled pilot study on 72 patients with OA symptoms was published in 1996. Patients under conventional treatment received 3,000 mg/day of niacinamide over 12 weeks. The global arthritis impact, measured by Visual Analog Scale (VAS), improved by 29 % in the verum group, with increased joint mobility by 4.5 degrees over controls. Treated patients also showed a decrease in erythrocyte sedimentation rate. The authors concluded that niacinamide may have a role in the treatment of OA by improving the global impact of OA, joint flexibility, and mitigating inflammation. Patients in the niacinamide group also reduced their use of anti-inflammatory medications by 13 % [14].

Several mechanisms have been suggested to explain the observed effects of nicotinates. Niacinamide has been shown to suppress cytokine-mediated induction of nitric oxide synthase in various cell types [15]. This results in decreased inflammation as reflected by the effects on interleukin-1-on chondrocytes.

The anti-inflammatory effect of the nicotinates is speculated to be connected to their effect on the immune system. An *in vitro* study where the niacin receptor Gpr109a was knockout in mice [16] there was a reduction of $Foxp3^+$ cells (regulatory T cells or Tregs), and resulted in production of IL-10 and IL-18 by $CD4^+$ T cells as well as the pro-inflammatory cytokine IL-17. In addition, CD 103^+ was unable to induce the differentiation of $CD4^+$ T cells into Tregs. *In vitro* addition of niacin to the heterozygous knockout led to incremental differentiation of $CD4^+$ T cells into Tregs. The authors concluded that niacin, in pharmacological doses, is likely to reach the colon and exert GPR109A-dependent effects on the T cell regulation pathway. These results suggest that niacin may help the activation of Tregs, leading to restoration of joint deterioration. Kenez et al., [17] showed that niacin and butyric acid, but not niacinamide, activate GRP109a *in vitro*. All these results suggest that nicotinates may be beneficial for patients with, or at risk of developing, OA. A number of animal models have been developed for investigating the onset of OA. However, due to the heterogenicity of OA (mild, moderate, or strong), there is a need to develop an appropriate animal model to mimic the level of severity of OA symptoms in humans. Post-traumatic OA models are the most widely used. The joint damage can be induced by micro-surgery [18], blunt force [19], injection of collagen in the Collagen Induced Arthritis (CIA) model [20], or chemically induced arthritis.

One animal model useful for investigating chronic nociceptive pain is the Monosodium Iodoacetate (MIA) rat or mouse model that has been found to be reproducible and mimicking OA pain in humans [21-25]. The objective of the present study is to evaluate the right dose of MIA to induce mild to moderate onset of OA-like symptoms in rats and suggest a model to evaluate the niacin and niacinamide impact on this condition. Three concentrations of MIA have been evaluated by measuring Dynamic Weight Bearing (DBW) capacity as an indicator of joint pain and by measuring joint diameter size as an indicator of inflammation. In addition, histopathological evaluation of the knee joint was performed to examine effects of the treatment on joint microscopic changes.

Materials and methods

Animals and induction of OA

Male Sprague Dawley rats were used in the study. Animals were 7-8 weeks old at the time of arrival. Animals were pair-housed in solid-bottom caging with corn-cob bedding equipped with an automatic watering valve, except during designated procedures. They received standard rodent chow (PMI Nutrition International Certified Rodent Chow No. 5CR4 with 14 % protein) and water (softened and purified by reverse osmosis

and exposed to ultraviolet light) *ad libitum*. For psychological/environmental enrichment, animals were provided with items such as chewing objects, nesting material, and hiding tunnels, except during designated activities. Veterinary care was available throughout the course of the study and animals were examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations were documented in the study records. Animals were acclimatized in the testing environment for at least 5 days before start of any experiment. OA was induced by a single intra-articular injection of Monosodium Iodoacetate (MIA) into the right hind knee of the rats given under isoflurane anesthesia. The day of MIA injection was considered as Day 0. Ten rats were used in each treatment group (Table 1). The Niacinamide dose was based on the maximal dose used in human [13] and the Niacin dose was chosen based on Salem and collegues [26]. This study was approved by Institutional Animal Care and Use Committee Approval (Testing Facility Study No. 5900813).

Table 1: Experimental design.

Groups	Group Treatment	TI Dose level (mg/kg)	TI Route, Volume	Dosing schedule	N
1	Naive / Niacinamide	400	10ml /kg	3 days	2
2	Naïve / Niacin	80	10ml /kg	3 days	2
3	Naïve / Vehicle	0	10ml /kg	Day -7 to Day 24	10
4	MIA 0.5 mg / vehicle	0	10ml /kg	Day -7 to Day 24	10
5	MIA 1.0 mg / vehicle	0	10ml /kg	Day -7 to Day 24	10
6	MIA 2.0 mg / vehicle	0	10ml /kg	Day -7 to Day 24	10
7	MIA 0.5 mg / Niacinamide	400	10ml /kg	Day -7 to Day 24	10
8	MIA 1.0 mg / Niacinamide	400	10ml /kg	Day -7 to Day 24	10
9	MIA 2.0 mg / Niacinamide	400	10ml /kg	Day -7 to Day 24	10
10	MIA 0.5 mg / Niacin	80	10ml /kg	Day -7 to Day 24	10
11	MIA 1.0 mg / Niacin	80	10ml /kg	Day -7 to Day 24	10
12	MIA 2.0 mg / Niacin	80	10ml /kg	Day -7 to Day 24	10

Detailing the experimental designs of the pilot study and the main study, stating concentrations of MIA and niacin/niacinamide, daily dosing schedule and numbers of animals used per group. *In vivo* DWB and joint diameter are assessed at baseline, days 3, 7, 14 abnd 24.

Measurement parameters

The BioSeb® Dynamic Weight Bearing (DWB) system was used to measure the weight bearing in the freely moving rats. The system consists of an arena box made out of a pressure-sensitive sensor mat on the bottom and an attached high-resolution camera on the top. A two-minute recording was done for each rat. Analysis of DWB data was done off-line using the BioSeb® software. The system automatically calculated the weight borne by each limb and the tail. Body weight was measured for each rat immediately before the DWB for each time of testing. The difference between percent weight bearing by the left hind limb (non-MIA side) and the right hind limb (MIA side) was used as the dependent variable as an indication joint pain (also referred to as DWB values). Joint measurements were done with a high precision micrometer (Kroeplin Laengenmesstechnik, 0-20 mm) to assess the MIA-induced inflammation. Rats were lightly restrained by hand and then the caliper placed medio-laterally in the right knee joint to measure the joint diameter. Histopathologic evaluation was performed in the right knee joints that were fixed and decalcified. In brief, knee joint samples were decalcified, embedded in paraffin wax and sectioned coronally. A single mid-section was taken at the level of the Anterior Cruciate Ligament (ACL) and stained with Safranin-O/Fast Green (SOFG). For each section, the four articular compartments (lateral and medial sides of both femoral condyle and tibial plateau i.e. FM-femur medial, FL-femur lateral, TM-tibia medial, TL-tibia lateral) were graded for OA disease severity by a veterinary pathologist using a Modified Mankin Score System. For each compartment, the following parameters were scored: structural changes (0-10), losss of SOFG staining (0-6), clones or cluster formation (0-3) and loss of chondrocytes (0-6). Summation of these scores yielded a 'composite score' for each compartment. Then a 'total composite score' was calculated by summation of all scores for the four compartments.

Dose formulations preparation

Monosodium-Iodoacetate was dissolved in physiologic saline (0.9% sodium chloride, USP) to obtain the desired concentrations. The dose volume was 0.025 mL/animal injected into the right knee joint only once. Niacinamide and niacin were also dissolved in the vehicle physiological saline at the desired concentrations and administered orally (PO).

Experimental design

The experimental design is described (Table 1). Before initiating the main experiment, a dose tolerability pilot experiment was performed in two groups (Group 1-2) of rats in order to examine the tolerability of niacinamide and niacin doses used in the main experiment. The dose levels of 400 mg/kg of niacinamide and 80 mg/kg of niacin were well tolerated in the rats without any clinical signs and thus were chosen for the for main study. In the main experiment, naïve rats were first randomized into treatment groups based on body weight, right knee joint diameter and their baseline DWB values. Afterwards, they received vehicle, niacinamide and niacin starting seven days before the MIA injection at the three different MIA concentrations (0.5 mg, 1 mg and 2 mg in 25 µL of saline). In total, ten groups of rats were used (Group 3-12 in (Table 1); one naïve control group that received vehicle, three control vehicle groups for each concentration of MIA, three treatment groups with 400 mg/kg dose of niacinamide for each concentration of MIA and three treatment groups with 80 mg/kg niacin for each concentration of MIA). The rats received the

supplementation by oral gavage, once daily from day 7 prior to MIA injection to day 24 post MIA injection (Table 1). Post MIA measurements for DWB and joint diameter for evaluation of pain and inflammation were performed on Days 3, 7, 14 and 24. At termination on Day 24, right knee joints were collected and used for histopathological evaluation.

Statistical analysis

Statistical analysis of the DWB and joint diameter data were performed using one-way Analysis of Variance (ANOVA) and Dunnett's post-hoc test for multiple comparions. Data were compared with the MIA control vehicle treated rats in each phase. Statistical analysis of the histopathology data was performed using the following method: A Kruskal-Wallis test was used in order to assess the overall group effect, and whenever found to be significant, group pairwise comparisons were performed using a two-sided Dunn's test. All statistical tests were conducted at the 5% significance level. For the pairwise comparisons, significance was reported at the 0.01% (****), 0.1% (***), 1% (**), and 5% (*) levels as compared to pre-treatement.

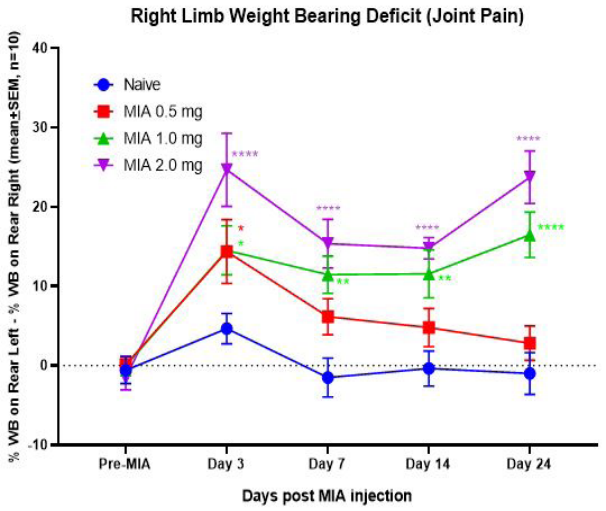

Figure 1: Time-course of MIA-induced joint pain in the rats. Comparison of % WB on rear left to % WB on rear right (Mean ± SEM) for naïve and MIA-treated rats showing statistically significant deficit in weight bearing in in the MIA rats compared with naïve rats. All significance at different days are compared to Pre-MIA.

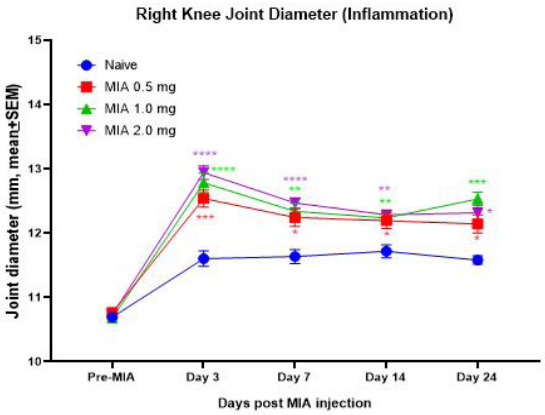

Figure 2: Time-course of MIA-induced joint inflammation in the rats. Comparison of joint diameter (Mean ± SEM) for naïve and MIA-treated rats showing joint diameters statistically significantly larger than naïve in all MIA groups starting from Day 3 to day 24. All significance at different days are compared to Pre-MIA.

Results

MIA dose-dependently induced pain, inflammation and microscopic changes in the injected knee joints in the rats

Joint pain was assessed in the rats using the Dynamic Weight Bearing (DWB) measurement system where a deficit in weight bearing in the right hind limb will indicate joint pain. Figure 1 shows a statistically significant deficit in weight bearing in all MIA groups compared to Naïve starting from Day 3, indicating joint pain perception at all three concentrations. The effect seems to be biphasic with an early peak at day 3 and a second increase at day 24 in the MIA 1.0 mg and MIA 2.0 mg groups until study end at Day 24, while in the MIA 0.5 mg group, the effect lessens progressively and is no longer statistically significant at Day 24. The diameter of the treated joint was statistically significantly larger than Naïve in all MIA groups starting from Day 3, indicating increased inflammation in all three treated groups. The effect persisted until study end at Day 24 (Figure 2). A statistically significant dose-dependency could not be shown. In comparison to the untreated animals, joint pathology scores in the treated animals in all groups was increased significantly at termination, day 24 (Figure 3). Both individual compartment scores and the total composite score of all four compartments showed that joint pathology were evaluated [22,27] and increased in a dose-dependent manner. Comparison between untreated, MIA-induced and MIA/Niacinamide treated images are shown and discussed in the (Figure 7).

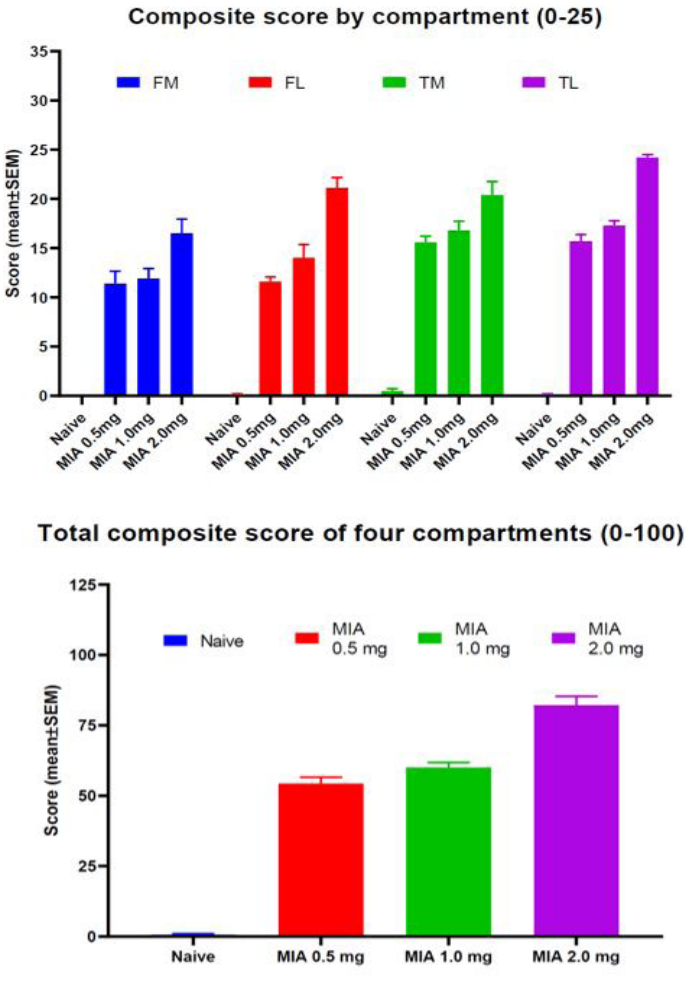

Figure 3: MIA-induced joint pathology at termination timepoint. Composite score by compartment and total composite score (Mean ± SEM) of naïve and MIA-treated rats showing that individual compartment scores and the total composite score of all four compartments showed that joint pathology increased in a dose-dependent manner in the MIA groups.

Niacinamide and niacin attenuated MIA-induced joint inflammation and induced microscopic changes in the injected knee joints in the rats without effects on DWB

Niacinamide effects on reducing joint diameter started at day 3 (Figure 4). These effects are significant for the MIA 2.0 mg/niacinamide group as compared to vehicle at day 3 and persist to day 14. On Day 7, statistical significance was also observed in the MIA 0.5mg/niacinamide group compared to vehicle. On Day 14, all three MIA dose groups showed statistical significance compared to their respective controls. On Day 24, the MIA 1.0mg/niacinamide group showed a statistical significant difference in joint diameter compared to vehicle as well.

No changes were found with niacin on reducing joint diameter at any of the MIA doses (Figure 5). Neither niacinamide nor niacin had a statistically significant effect on the perception of joint pain compared to vehicle as measured by DWB (Figure 6).

Niacinamide reduced the low dose MIA-induced joint microscopic changes

Microscopic evaluation showed that the composite joint score of microscropic changes induced by MIA, by group, are consistent with OA. In the (Figure 7), a concentration-dependent increase in severity of these changes, which was characterized by higher composite scores, was noted with increasing MIA concentrations as previously reported in (Figure 3). The lateral tibial plateau was generally most affected by OA-related changes compared to the other compartments. The median composite scores for the 0.5 mg MIA/niacinamide group was the lowest across all MIA-treated rats, mostly due to a reduction in histopathologic changes parameters' scores within the femoral compartment. There was no effect of niacin (80 mg dose) on composite parameter scores at any MIA level compared to those animals treated with MIA alone. There was no notable effect of niacinamide at MIA concentrations of 1.0 mg and 2.0 mg on composite parameter scores compared to those animals treated with MIA alone. However, at 0.5 mg MIA in presence of Niacinamide, noticeable amelioration can be observed (Figure 7).

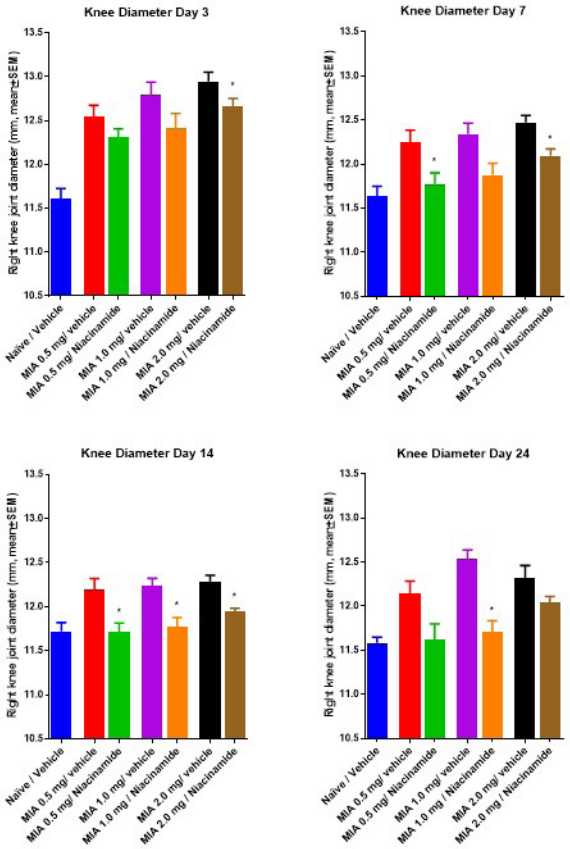

Figure 4: Effects of Niacinamide on MIA-induced joint inflammation. Comparison charts of right joint diameters of MIA-treated rats either treated with vehicle or with niacin at different concentrations (Mean ± SEM) at day 3, 7, 14 and 24 showing statistically significant effects for the MIA 2.0 mg/niacinamide group at day 3 and for the MIA 0.5mg/niacinamide group at day 7 as compared to vehicle.

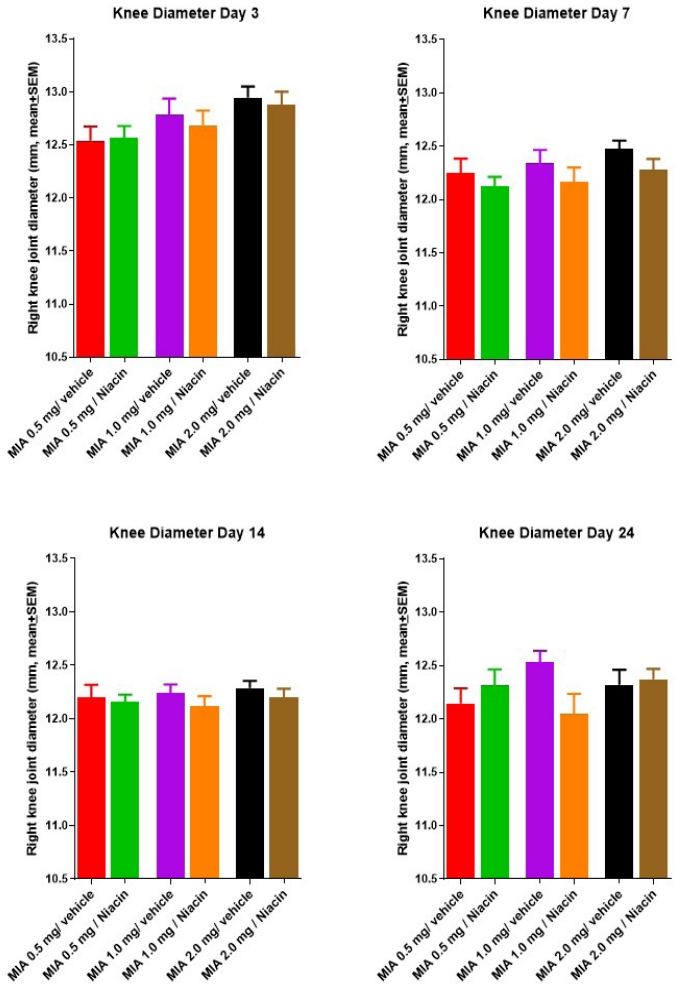

Figure 5: Effects of Niacin on MIA-induced joint inflammation. Comparison charts of right joint diameters of MIA-treated rats either treated with vehicle or with niacin at different concentrations (Mean ± SEM) at day 3, 7, 14, and 24 showing no statistically significant differences.

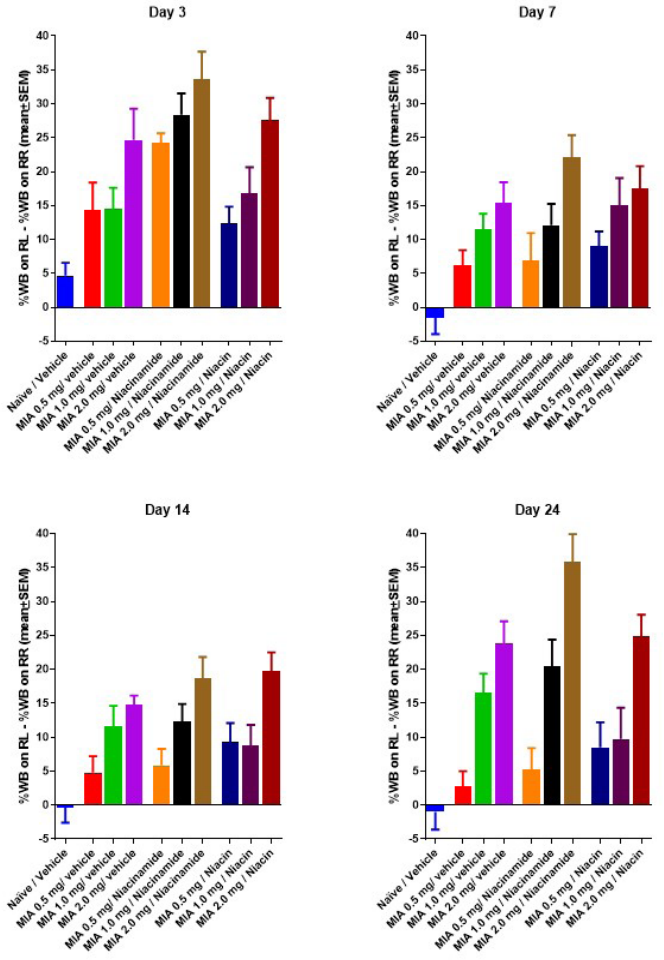

Figure 6: Effects of niacinamide and niacin on MIA-induced joint pain. Comparison charts of differences in % DWB of naïve rats treated with vehicle and MIA-treated rats either treated with vehicle or with niacinamide or niacin at different concentrations (Mean ± SEM) at day 3, 7, 14, and 24 showing that neither niacinamide nor niacin had a statistically significant effect on the perception of joint pain compared to vehicle.

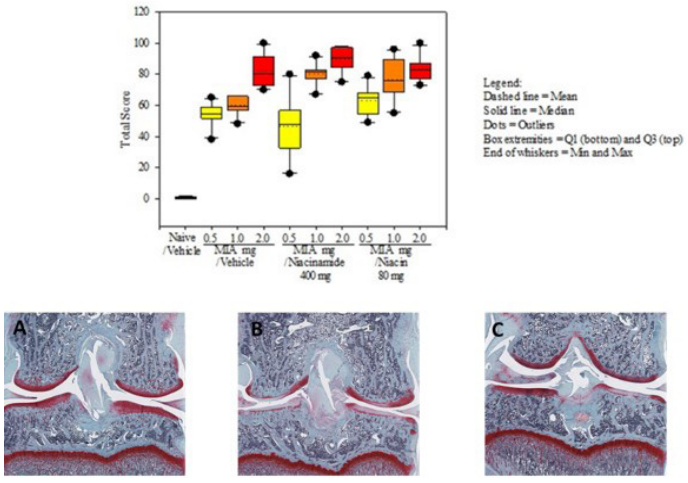

Figure 7: Effects of niacinamide and niacin on MIA-induced joint pathology. Total score of microscopic changes in the joint of naïve rats treated with vehicle and MIA-treated rats either treated with vehicle or with niacinamide or niacin at different concentrations (Mean±SEM) at termination point showing a concentration-dependent increase of microscopic changes induced by MIA. A representation of the SOFG staining of the right knee joint, coronal section at the level of the Anterior Cruciate Ligament (ACL), 30x magnification is shown in the lower panel for naïve (A), 0.5 mg MIA treated (B) and 0.5 mg MIA/Niacinamide treated (C) animals.

Discussion

Results showed a dose-dependent induction of OA-like effects on the knee joint pain and inflammation of the rats. Effects were seen with all three concentrations of MIA (0.5 mg, 1.0 mg, and 2.0 mg) following a single intra-articular injection.

Rats responded to the injection with changes in weight bearing indicating an increase in pain perception. The extent of these weight bearing deficits was dependent on the concentration of the injected MIA, supporting results from previous investigations in the mouse [23] and rat [24]. Neither uptake of niacinamide nor niacin significantly affected the DWB changes induced by MIA, indicating that neither substance affected pain. However, the initial swelling of the joint as indicated by joint diameter measuring on day 3 was reduced by niacinamide at day 3 reaching statistical significance at day 7 in Group 7 (0.5 mg MIA) and Group 8 (1.0 mg MIA), suggesting that niacinamide effects in mitigating inflammation are reached sooner under lower severity conditions (0.5 mg MIA). These effects persisted to day 24. This suggests that mild to moderate OA-like symptoms of joint swelling may be ameliorated by nutritional supplementations such as niacinamide in this model. The effect of niacinamide on MIA-induced joint swelling may be the result of reduced inflammation as previously reported for the niacinamide mechanism of action [15,16]. Histopathological investigation confirmed and supported the clinical observation, showing microscopic changes of the joint consistent with OA after 24 days, the earliest time point for this assessment. The effect of niacinamide on the MIA rat model (knee joint diameter) became visible after day 3 and significant at day 7. Previous models [28-30] reported results only after 28-40 days. In addition, in our testing, the best response to niacinamide was seen at 0.5 mg MIA, an optimal dose compared to the 0.3 mg not leading to significant histological changes or an intervention response [31]. Higher doses previously used led to a pharmacoligal response to pain [25]. In our study, we used 1 and 2 mg doses to evaluate also a response to inflammation. All doses used led to a mitigation in the inflammation. The highest MIA dose (2 mg) showed an effect in mitigating inflammation by niacinamide as early as day 3 and the lowest dose showed a response at day 7. All the doses responded to the niacimade pre-treatement at day 14. After 24 days, only the 1 mg MIA dose is still significant, suggesting perhaps multi-phasic progression in the inflammation and possibly different effects/mechanisms for nicotinates in mitigating inflammation based on the severity of the OA-related symptoms. In deed, several mechanisms of nicotinate intervention have been suggested [15-17]. The Collagen-Induced Arthritis (CIA) rat model was developed for investigations on rheumatoid arthritis, a more severe condition involving induction of synovial hyperplasia and inflammation; it only shows the first signs of onset of arthritis after 10 days and reaches its peak after 21 days [20]. The Partial Medial Meniscectomy Tear (PMMT) method is a surgical method that induces OA-like degenerative effects by damaging the medial meniscus; it only shows OA symptoms 8 weeks after surgery [18]. The MIA model used in this study shows significant OA symptoms as early as 7 days after induction. This model with a 0.5 mg MIA dose is useful for assessing early effects of supplements on the onset of mild to moderate OA-like symptoms and provides an improvement over previous models.

Conclusion

The 0.5 mg MIA rat model for OA used in this study has shown a dose-dependent effect of MIA on joint pain and inflammation

as supported by both clinical observation of changes in DWB and joint diameter as well as by histopathological observation. The model responded to substances with proven effect against OA and RA such as niacinamide. This indicates that the model may be useful in the investigation of human subjects with mild to moderate severity of onset of OA symptoms or healthy subjects at-risk. In addition, this model is useful to assess the effects of nutraceutical supplements appropriate to use among this demographic.

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