Biochemistry and Biophysics Reports 32 (2022) 101341

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Maackia amurensis seed lectin (MASL) ameliorates articular cartilage destruction and increases movement velocity of mice with TNFα induced rheumatoid arthritis

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ARTICLE INFO

Keywords:
Arthritis
Rheumatoid arthritis
TNF
Maackia
Lectin
MASL

ABSTRACT

Up to 70 million people around the world suffer from rheumatoid arthritis. Current treatment options have varied efficacy and can cause unwanted side effects. New approaches are needed to treat this condition. Sialic acid modifications on chondrocyte receptors have been associated with arthritic inflammation and joint destruction. For example, the transmembrane mucin receptor protein podoplanin (PDPN) has been identified as a functionally relevant receptor that presents extracellular sialic acid motifs. PDPN signaling promotes inflammation and invasion associated with arthritis and, therefore, has emerged as a target that can be used to inhibit arthritic inflammation. *Maackia amurensis* seed lectin (MASL) can target PDPN on chondrocytes to decrease inflammatory signaling cascades and reduce cartilage destruction in a lipopolysaccharide induced osteoarthritis mouse model. Here, we investigated the effects of MASL on rheumatoid arthritis progression in a TNFα transgenic (TNF-Tg) mouse model. Results from this study indicate that MASL can be administered orally to ameliorate joint malformation and increase velocity of movement exhibited by these TNF-Tg mice. These data support the consideration of MASL as a potential treatment for rheumatoid arthritis.

1. Introduction

Over 10% of men and 18% of women over 60 years old suffer from destruction of joint cartilage and underlying bone caused by osteoarthritis. In addition, up to 1% of the world population suffer from bone and cartilage destruction caused by rheumatoid arthritis. Options for arthritis treatment include disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate, steroids, non-steroidal anti-inflammatory drugs (NSAIDs), and biologics. However, these drugs can adversely affect immune, kidney, liver, gastrointestinal, and cardiovascular systems [1-3]. New approaches are needed to treat these conditions.

Protein glycosylation modifications are associated with a variety of inflammatory diseases. In particular, alterations α-2,6 and α-2,3 sialic acids on chondrocytes are associated with arthritic progression and cartilage damage [4,5]. Endogenous lectins (galectins) target these glycoprotein carbohydrate motifs to affect cell extracellular matrix production, migration, growth, and inflammation. For example, galectins with affinity to α-2,3 sialic acid motifs presented by receptors on chondrocytes promote arthritic joint and bone destruction [6,7].

Glycoprotein receptor activation stimulates the NFκB pathway to induce inflammation that leads to arthritic conditions. This NFκB activation induces cytokine production, particularly TNFα and interleukins (e.g. IL6 and IL17), which initiate JAK-STAT and Src kinase signaling pathways that cause cartilage destruction [6,8,9]. PDPN has been identified as a receptor that galectins target to initiate this process [10-13]. PDPN is transmembrane protein that presents a highly *O*-glycosylated extracellular region containing α-2,3 sialic acids. PDPN signaling promotes inflammation and invasion associated with arthritis and, therefore, has emerged as a target that can be used to inhibit and arthritic inflammation [3].

Maackia amurensis seed lectin (MASL) can target PDPN on

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https://doi.org/10.1016/j.bbrep.2022.101341
Received 12 August 2022; Received in revised form 29 August 2022; Accepted 30 August 2022

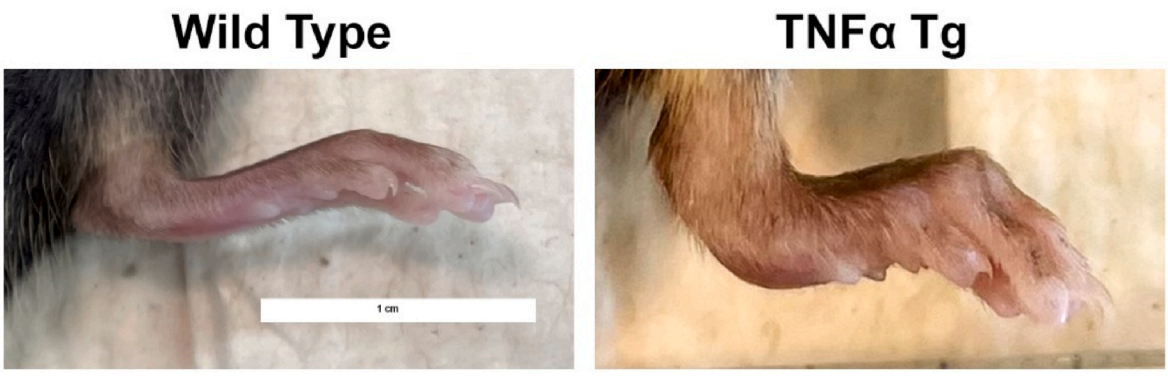

Fig. 1. Joint malformation in TNFα transgenic (TNF-Tg) mice: Wild type and TNF-Tg mice were maintained for 147 days, and rear paws were photographed as indicated (bar = 1 cm).

chondrocytes and to decrease inflammatory signaling cascades [12]. MASL treatment was found to inhibit NFκB nuclear translocation and IL6 expression in arthritic human chondrocytes. In addition, weekly oral MASL administration was found to reduce cartilage extracellular matrix degradation in lipopolysaccharide induced arthritic mice compared to placebo controls [12].

We hypothesized that MASL can be administered orally to inhibit arthritic inflammation and joint destruction caused by rheumatoid arthritis. We utilized a TNFα transgenic (TNF-Tg) mouse model of arthritis for this work. These mice are widely utilized to model spontaneous progressive inflammatory and erosive arthritis with a well defined and clinically relevant etiology [14-18]. Results from this study indicate that MASL can be administered orally to ameliorate joint malformation and increase velocity of movement exhibited by these TNF-Tg mice.

2. Materials and methods

2.1. Mouse maintenance

Mouse experimental procedures were performed under Rowan University IACUC approved protocols according to applicable federal and state regulations. TNF-Tg mice were maintained as hemizygotes in a C57BL/6 background as described [14,19-23]. Homozygous wild type and hemizygous TNF-Tg mice were used for experiments after identification by PCR as previously described [22]. Forty five days after birth, MASL (Sentrimed) was orally administered as described [12,24] at a final dose of 100 mg/kg mixed 1:1 (V:V) in a total volume of 0.2 ml with Chocolate Ensure (Ensure) three times per week. Ensure was administered without MASL as a placebo. Fourteen mice were included in this study including 5 wild type (3 female and 2 male), 4 placebo treated TNF-Tg (2 females and 2 males), and 5 MASL treated TNF-Tg (2 female and 3 male) mice.

2.2. Mouse morphology assessment

During feeding, each mouse was assessed for progression of arthritic pathological features as described [14,19-23]. Each paw was graded from 0 to 4 based on the level of joint redness, edema, inflammation, and joint deviation. Values were summed to calculate a total assessment score from 0 to 16, with 0 being the lowest and 16 indicating the most aggressive disease criteria. Mice weights were assessed about every two weeks.

2.3. Locomotion activity assessment

Mouse movement velocity and distance traveled during a 30-min time period were measured in an open field arena (27.3 cm x 27.3 cm) equipped with Activity Monitor Version 6.02 software (Med Associates, Inc). Velocity and distance traveled were measured by the breaking of photobeams. Each analysis was performed at 2-week intervals starting at 114 days after birth as described [25]. Movement velocity was defined as the average speed of each ambulatory episode and distance traveled was defined as the overall ambulatory distance traveled over the 30-min time period.

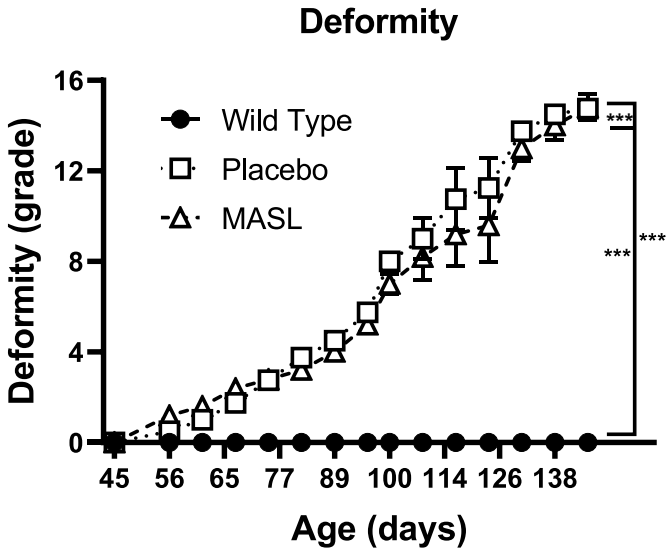

Fig. 2. Progressive joint deformity in TNF-Tg mice treated with MASL or placebo over time: Wild type and TNF-Tg mice were maintained for 45 days before MASL (100 mg/kg) or placebo was orally administered to TNF-Tg mice 3 days per week. Joint deformity was measured on a scale of 0–16 at the indicated time points. Data are shown as mean ± SEM.

2.4. Euthanization and tissue processing

Mice were euthanized by CO2 asphyxiation followed by cervical spine dislocation and prepared for dissection as described [26]. Ankles, heart, kidney, liver, lungs, and small intestine were harvested and fixed in 10% neutral buffered formalin and processed for histology and IHC to detect PDPN as described [12,24,27].

3. Results

3.1. TNF-Tg mice develop rheumatoid arthritis symptoms and morphology

We utilized a TNFα transgenic (TNF-Tg) mouse model to investigate the effects of MASL on rheumatoid arthritis progression. The heterozygous TNF-Tg mice develop joint malformation indicative of rheumatoid

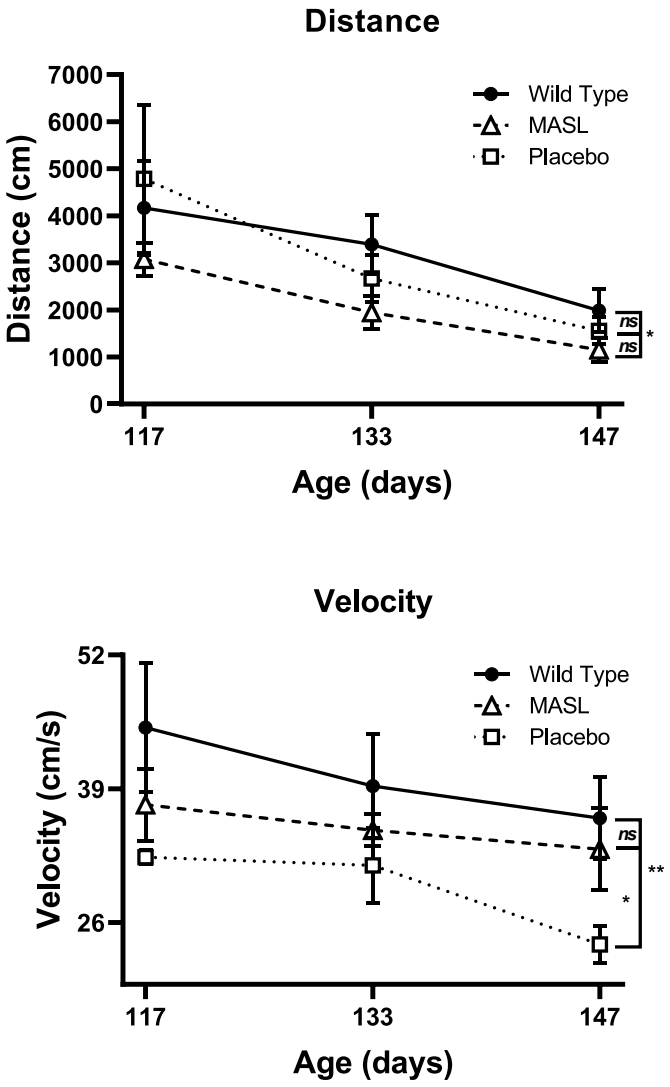

Fig. 3. Effect of MASL on TNF-Tg mouse movement: Wild type and TNF-Tg mice were maintained 45 days before MASL (100 mg/kg) or placebo was orally administered to TNF-Tg mice 3 days per week. Distance was measured as cm traveled during a 30 min time period, while velocity was measured as cm traveled per second during movement, at indicated time points. Data are shown as mean ± SEM with $p < 0.01$ and $p > 0.05$ indicated by double asterisks and ns by ANOVA, respectively.

arthritis. In contrast, wild type litter mates display normal morphology and are used as controls for this study. This is evident by joint redness, edema, inflammation, and joint deviation in adult transgenic mice, but not wild type littermates, as shown in Fig. 1.

3.2. MASL administration decreases TNF induced arthritic morphology

Wild type and heterozygous TNF-Tg were maintained 45 days before MASL (100 mg/kg) or placebo was orally administered to TNF-Tg mice 3 days per week and joint deformity was measured over time as shown in Fig. 2. Progressive disease in TNF-Tg mice started at about 50 days after birth. This gradually progressed to grade 4.5 ± 0.29 in placebo treated mice, and slightly lower to grade 4.0 ± 0.32 in MASL treated mice by 89 days after birth (mean ± SEM). Morphological symptoms of disease progression accelerated to grade 10.8 ± 1.4 and 9.2 ± 1.4 by day 117 for placebo and MASL treated TNF-Tg mice, respectively. Morphological symptoms plateaued at grade 14.8 ± 0.25 and 14.8 ± 0.58 for placebo and MASL treated TNF-Tg mice at 145 days after birth. In contrast to these TNF-Tg mice, disease symptoms were not seen in wild type littermates (grade 0).

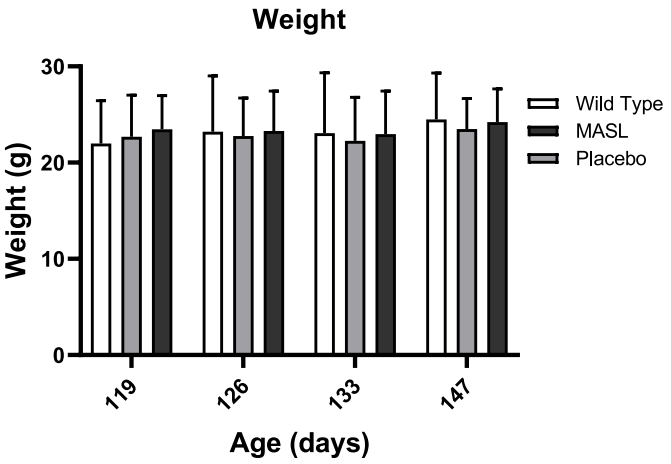

Fig. 4. Weight of wild type of TNF-Tg mice: Wild type and TNF-Tg mice were maintained 45 days before MASL (100 mg/kg) or placebo were orally administered to TNF-Tg mice 3 days per week. Weight was measured at indicated time points. Data are shown as mean ± SEM.

3.3. MASL administration increases TNF-Tg mice movement velocity

A general decrease in mouse movement was seen with age as shown in Fig. 3. Wild type traveled an average distance of 4168 ± 1006 cm, 3389 ± 637 cm, and 1986 ± 458 cm (mean ± SEM) in 30 min at 117, 133, and 147 days after birth, respectively. Placebo treated TNF-Tg mice traveled and average distance of 4785 ± 1568 cm, 2668 ± 502 cm, and 1556 ± 287 cm in 30 min at 117, 133, and 147 days after birth, respectively, which was similar to wild type mice ($p > 0.05$ by ANOVA). MASL treated TNF-Tg mice traveled and average distance of 3076 ± 353 cm, 1946 ± 355 cm, and 1152 ± 259 cm in 30 min at 117, 133, and 147 days after birth, respectively, which was less than wild type mice ($p < 0.05$ by ANOVA) but similar to placebo treated TNF-Tg mice ($p > 0.05$ by ANOVA).

In contrast to distance traveled, wild type mice moved at a significantly higher velocity than TNF-Tg mice treated with placebo as shown in Fig. 3. Wild type mice traveled an average of 45 ± 6.3 cm/s, 39 ± 5.0 cm/s, and 36 ± 4.0 cm/s (mean ± SEM) at 117, 133, and 147 days after birth, respectively. Placebo treated TNF-Tg mice traveled an average of 32 ± 0.7 cm/s, 32 ± 3.6 cm/s, and 24 ± 1.8 cm/s at 117, 133, and 147 days after birth, respectively. This was significantly slower than wild type mice ($p < 0.01$ by ANOVA). MASL treated TNF-Tg mice traveled an average of 37 ± 3.5 cm/s, 35 ± 1.6 cm/s, and 33 ± 4.0 cm/s at 117, 133, and 147 days after birth, respectively. This was significantly faster than placebo treated TNF-Tg mice ($p < 0.05$ by ANOVA) and similar to wild type mice ($p > 0.05$ by ANOVA).

3.4. MASL administration does not cause deleterious effects on mouse weight, behavior, or organ tissue morphology

MASL did not affect wild type or TNF-Tg mouse weight. Adult mice weighed an average of 22–25 g throughout this investigation as shown in Fig. 4. Mouse weight was not affected by MASL treatment or TNF-Tg expression ($p > 0.05$ by ANOVA). MASL administration did not affect mouse morphology or behavior. Histochemical examination found no effects of MASL administration on heart, kidney, liver, lung, or small intestine tissue morphology as shown in Fig. 5.

3.5. MASL administration decreases TNF induced ankle cartilage destruction

Examination of ankle tissue by IHC found that PDPN was expressed

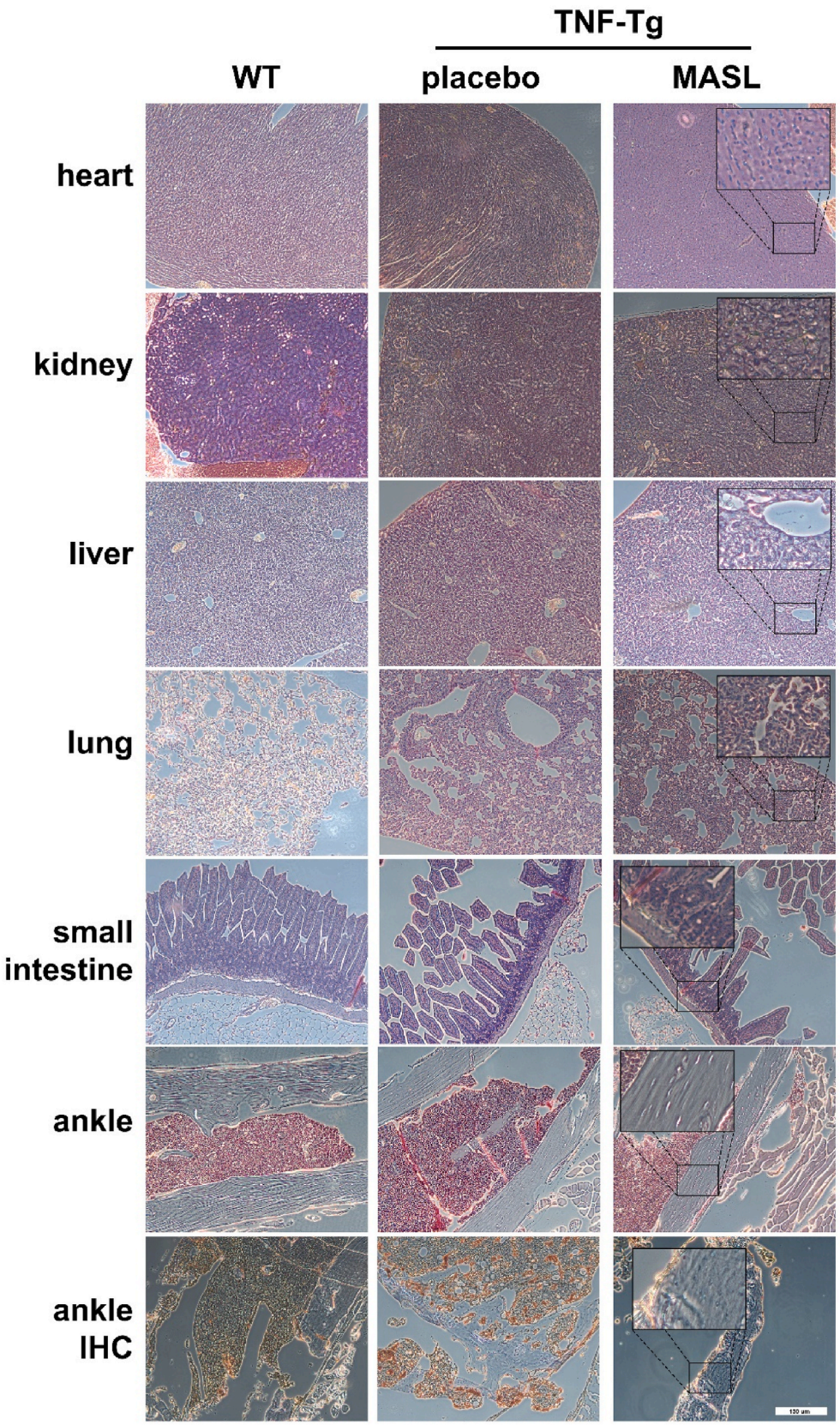

Fig. 5. Tissue morphology in wild type and TNF-Tg mice: Wild type and TNF-Tg mice were maintained 45 days before MASL (100 mg/kg) or placebo was orally administered to TNF-Tg mice 3 days per week for 15 weeks before animals were sacrificed. Heart, kidney, liver, lung, small intestine, and ankles were examined by H&E staining, and PDPN expression in ankle tissue was examined by IHC, as indicated (bar = 130 μm).

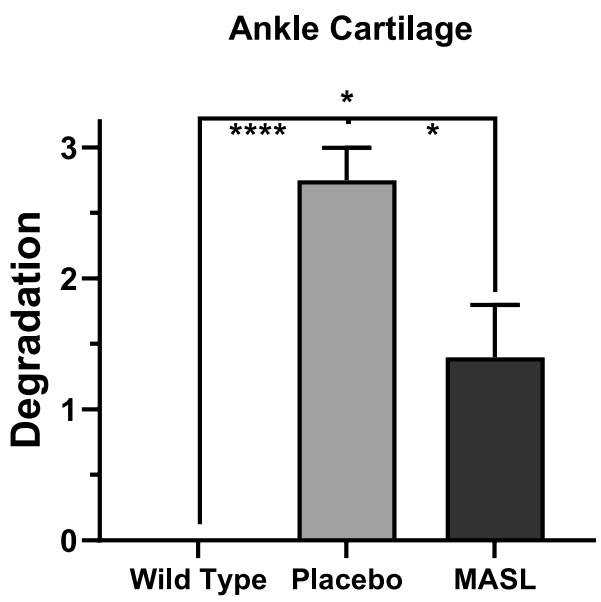

Fig. 6. Effect of MASL on ankle tissue structure: Wild type and TNF-Tg mice were maintained 45 days before MASL (100 mg/kg) or placebo was orally administered to TNF-Tg mice 3 days per week for 15 weeks before animals were sacrificed. Ankles were examined by H&E staining. Cartilage destruction was graded on a scale of 0–4 and shown as mean ± SEM with $p < 0.0001$ and $p < 0.05$ indicated by quadruple and single asterisks by *t*-test, respectively.

in cartilage and osteocytes as shown in Fig. 5. This is consistent with previous reports [28-30], and results from recent studies indicating that MASL can target PDPN in order to ameliorate arthritic bone and cartilage destruction [12]. Indeed, in contrast to general organ and tissue morphology, MASL did appear to affect bone cartilage morphology as shown in Fig. 5. TNF-Tg mice displayed thinner ankle bone associated cartilage layers than wild type mice. This thinning was less evident in MASL treated mice compared to controls. Cartilage degradation quantitated on a scale of 0–4 found a 2 fold difference between MASL and placebo treated TNF-Tg mice ($p < 0.05$ by *t*-test) as shown in Fig. 6.

4. Discussion

Maackia amurensis has been used as a medicinal plant for several centuries. Decoctions are used to treat inflammatory conditions including arthritis [31-34]. MASL is an extremely abundant component in these extracts. MASL can survive digestion and has been found to inhibit viral infection, cancer, and inflammation, and arthritis progression [12,24,35-38].

MASL targets extracellular receptors that present α-2,3-sialic acid moieties [39-41]. Some examples of these receptors include mucin 1 (Muc1), mucin 16 (Muc16), Leukosialin (CD43), Angiotensin converting enzyme 2 (ACE2), and podoplanin (PDPN). These receptors mediate signaling events that control a variety of processes including vascular cellular growth, immune cell activity, and inflammation [13,42-46].

Addition of α-2,3 sialic acid residues on chondrocyte receptor proteins are associated with arthritic progression and cartilage damage. Endogenous lectins (galectins) target these motifs to promote arthritic joint and bone destruction [6,7]. MASL can target these receptors, exemplified by PDPN, on chondrocytes to decrease inflammatory signaling cascades that lead to this arthritic progression. For example, 1 h treatment with 400 nM MASL significantly inhibited NFκB nuclear translocation in arthritic human chondrocytes, and decreases IL6 expression by 2 fold compared to parallel control cells [12].

Here, we investigated the effects of MASL on TNF induced rheumatoid arthritis a mouse model. Recent investigations found that weekly oral MASL administration (50 mg/kg) can reduce cartilage extracellular matrix degradation in LPS induced arthritic mice compared to placebo controls [12]. In contrast to a focal LPS insult generating osteoarthritis, insult was constant in these TNF-Tg mice. Therefore, mice were treated thrice weekly (100 mg/kg), to meet this persistent challenge. MASL treated TNF-Tg mice showed a slight decreased progression of joint malformation compared to placebo controls. However, malformation in both MASL and placebo treated mice plateaued at similar levels by 145 days after birth. MASL did not significantly affect the distance traveled by these TNF-Tg mice. However, MASL treatment did significantly increase TNF-Tg mouse movement velocity. MASL treated TNF-Tg mice traveled over 25% faster than placebo controls at 147 days after birth, suggesting a protective effect on motility in advanced arthritis stages. MASL administration also appeared to ameliorate ankle bone cartilage degradation by about 2 fold compared to placebo controls. As previously reported, MASL administration caused no deleterious effects on mouse weight, behavior, or organ and tissue morphology [12,24].

Taken together, these data suggest that MASL holds potential as a natural antiarthritic agent. Recent studies indicate the MASL exerts pleiotropic effects on a variety of inflammatory signaling cascades [37,38]. Although not examined in this study, future experiments could examine potential interactions between MASL and antioxidants, mitochondria, and gap junction proteins which play roles in cartilage maintenance [47-49]. The approach utilized for this study would be well suited to investigate multiple MASL dose regimens, and its potential for combination therapy with disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate, steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), and biologics that target JAK kinase and cytokines [13].

Declaration of competing interest

GSG has board membership, intellectual property, and ownership in Sentrimed, Inc. which is developing agents that target PDPN to treat diseases including cancer and arthritis. Other authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Kelly L. Hamilton: designed and performed experiments. **Amanda A. Greenspan:** designed and performed experiments. **Alan J. Shienbaum:** performed histology and analyzed data. **Bradford D. Fischer:** designed and performed experiments, Formal analysis, and contributed to manuscript writing. **Andrea Bottaro:** Conceptualization, designed, and performed experiments, Formal analysis, and contributed to manuscript writing. **Gary S. Goldberg:** Conceptualization, designed experiments, analyzed and interpreted data, and wrote the manuscript. All authors reviewed the article for accuracy and approval.

Acknowledgements

We are grateful to Christopher M. Laugier (Rowan University) for assistance with mouse feeding. This study was supported in part by funding from the Camden Health Research Initiative to GSG and AB, and NIH grant CA235347 to GSG.

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