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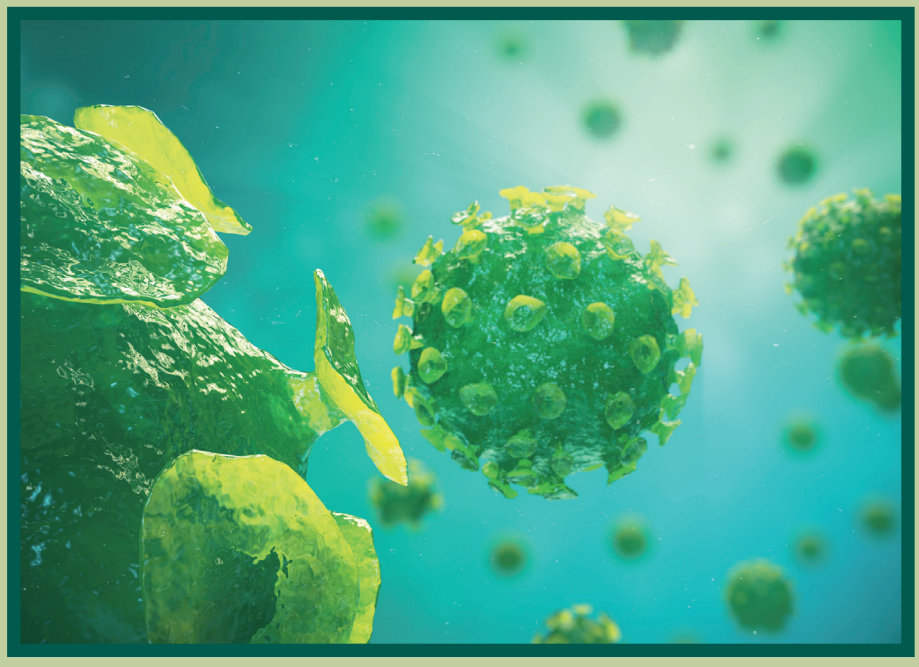

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ORIGINAL ARTICLE

Effect of probiotic on serum cytokines and matrix metalloproteinases profiles during monoiodoacetate-induced osteoarthritis in rats

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ABSTRACT

BACKGROUND: Cytokines (CKs) and matrix metalloproteinases (MMPs) play a major role in the pathogenesis of osteoarthritis (OA). The recent studies showed the effect of probiotics (PBs) on inflammatory processes during OA, but available results are limited and conflicted. In this study, we investigated the effect of alive probiotic on the levels of CKs (interleukins [ILs] IL-1β, IL-4, IL-10, IL-12 p40, tumor necrosis factor [TNF]-α, interferon [IFN]-γ) and MMPs (MMP-1, MMP-2, MMP-3, MMP-8) in rats with monoiodoacetate-induced OA.

METHODS: We used a single injection of monoiodoacetate through the infrapatellar ligament of nonlinear male rats to start OA model (1st day of the experiment). Therapeutic groups got an intragastric feeding of PB composition ("Symbiter" O.D. Prolisok, Kyiv, Ukraine) from 8th to 21st. Sampling (serum, knee joint) was provided on the 30th day. The pathophysiological aspects of OA was described by histology assessment of knee cartilage. The levels of CKs and MMPs were measured in serum by enzyme-linked immunosorbent assay.

RESULTS: OA model caused a significant increasing the levels of pro-inflammatory CKs (IL-1β, TNF-α, IFN-γ), and all studied MMPs, while IL-4 and IL-10 were decreased, comparing to group without OA (except IL12 p40, no significance changes in all groups). An application of the PB had positive effect on the levels of CKs and MMPs.

CONCLUSIONS: The use of the PBs has anti-inflammatory effect during experimental OA. Thus, action of PB is promising and further investigations are waited with interest.

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KEY WORDS: Inflammation; Models, animal; Osteoarthritis; Cytokines; Matrix metalloproteinases; Probiotics.

Osteoarthritis (OA) is the most common deep-rooted and, so far reckoned, as irretrievable disorder of the musculoskeletal system. OA is classically thought to be a non-inflammatory arthropathy involving onward degeneracy of cartilage and bone remodeling. However, some studies have consistently demonstrated a degree of inflammation at all stages of the disease process.[1] Indeed, cytokines (CKs), chemokines, and other inflammatory mediators are produced locally by the synovium and chondrocytes and are detectable in OA synovial fluid.[2, 3]

Changes observed in OA include cleavage of cartilage macromolecules with subsequent loss of it, a variable degree of synovial inflammation that are attributed to elaborate system of biochemical factors, among them proteinases.[4] It was revealed that cytokines which are connected to OA pathogenesis include tumor necrosis factor (TNF)-α,

matrix metalloproteinases (MMPs), interleukin (IL)-1, IL-6, other common γ-chain cytokines such as IL-2, IL-7, IL-15, and IL-21, and chemokines.[5, 6] Loss of cartilage activates chondrocytes and causes an out flux of collagenases, like MMPs, from matrix. Such reconfiguration leads to local initiation of the inflammatory reaction. The opposite action belongs to another group of cytokines like IL-4, IL-10, and IL-13 known as anti-inflammatory. Nevertheless, the functions and signaling pathways of these proteins in the pathogenesis of OA are still not clearly described.[7, 8] As OA is a debilitating disease with no cure and limited treatment options, there is a need in implementation of new therapeutic strategies. By this with fewer side effects of new therapies are desperately needed.

Whereas there is no flawless cure, only alleviation of pain and symptoms relief treatment settings such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), and glucosamine sulfate exist.[9] It was shown that the anti-inflammatory and anesthetic actions of NSAIDs resulting from the suppression of prostaglandin synthesis.[10] However, NSAIDs are connected to a broad variety of adverse effects such as gastric ulceration, renal failure, and lengthened bleeding times.[11] Consequently, a great solution will be to search for new and efficient pharmacological agents and biotechnological products to treat OA. It is now clear that the microbiome exerts a wide range of physiological functions in living organisms: regulation of cell and humoral immunity, intestinal mucosal state, synthesis and/or metabolism of the spectrum of compounds (vitamins, fats, fatty acids, bile acids, some essential amino acids).[12-15] For this reason, the role of microbiota is considered crucial in a wide spectrum of diseases.[16]

The recent studies declared the influence of probiotics (PBs) on inflammatory processes during OA, but available results are confined and controversial.[17-21] Several studies notified that probiotic treatment had been found to promote bone metabolism, decrease pain and inflammatory responses of age-related musculoskeletal diseases, including OA.[22, 23] Oral supplementation of *Lactobacillus casei* (a probiotic) alone or together with type II collagen (CII) and glucosamine (GS) (a promising prebiotic) was given to arthritic rats during monoiodoacetate-induced model of OA (MIA-OA). *L. casei* manifested a synergistic action with CII and GS, effectively reducing pain, cartilage erosion, and lymphocyte infiltration greater than the treatment with GS and CII alongside or separately. Coadministration also reduced the levels of various pro-inflammatory CKs (IL-1β, IL-2, IL-6, IL-12, IL-17, TNF-α, and interferon-γ (IFN-γ) and MMPs (-1, -3, and -13), while up-regulating anti-inflammatory CKs (IL-4 and IL-10).[24] Similar studies of PBs on collagen-induced arthritis in rats, a standard model of rheumatoid arthritis, have produced results comparable to those of NSAIDs.[17, 25] Suggesting that, irrespective of the underlying mechanism of joint inflammation, PBs may be beneficial.

Our previous works showed promising anti-inflammatory effect of live symbiotic biomass which contains 14 strains of microorganisms belonging to 10 species: *Bifidobacterium bifidum, B. longum, Lactobacillus acidophilus, L. delbrueckii, L. helveticus, Propionibacterium freudenreichii, P. acidipropionici, Lactococcus lactis, Acetobacter aceti, Streptococcus salivarius*.[26, 27] In the present study, we investigate whether the PB influence on profile of CKs and MMPs during MIA-OA in rats.

Materials and methods

Animals and experimental protocol

This study was carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the general ethical principles of animal experiments, approved by the First National Congress on Bioethics Ukraine (September 2001). The rats were kept in collective cages in controlled conditions of temperature (22±3 °C), light (12 h light/ dark cycle) and relative humidity (60±5%). The animals were fed laboratory chow and tap water ad libitum.

Initially, forty rats were randomly distributed into four groups (Figure 1). We used a single injection of MIA through the infrapatellar ligament of nonlinear male rats to start OA model.[28] Therapeutic groups got an intragastrical feeding of PB ("Symbiter", O.D. Prolisok) from 8th to 21st days of the experiment in a dose of 140 mg/kg dissolved in water. Blood sampling was provided on the 30th day. Knee joints of hind limb were fixed in 10% formalin and stored less than 1 month.

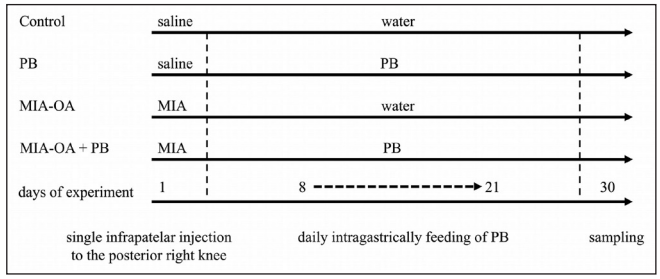

Figure 1.—Design of the experimental model. Arrows demonstrate experimental groups of animals (N.=10) and the manipulation within these groups.

Histological assessment

Knee joints removed from formalin and then decalcified in rapid decalcificator (Kaltek, Padua, Italy). Samples prepared in the histoprocessor of the carousel type "STP-12". Station EC-350 used to fill the paraffin blocks. Rotary microtubule HM-340E prepared histological sections. Device Robot-Stainer HMS-740 (all MICROM, Germany) colored the histological preparations with hematoxylin and eosin (G & E).[29] The microphotographs (magnification × 200) made by microscope Axio Cam MRc5 (Carl Zeiss, Oberkochen, Germany).

ELISA analysis

The levels of studied parameters (IL-1β, IL-4, IL-10, TNF-α, IFN-γ, MMP1, MMP2, MMP3, MMP8), were measured in serum by enzyme-linked immunosorbent assay (Biotrak ELISA System, GE Healthcare, Chicago, IL, USA), using manufacturer recommendations.

Statistical analysis

Mathematical calculations performed by using SPSS-20 software (IBM, USA). All data in this study were expressed as means±standard deviation (M±SD) or %. Continuous variables with parametric distribution were analyzed using Analysis of Variance (ANOVA) and if the result were significant, a *post-hoc* Tukey Test was performed. The difference between groups was defined to be statistically significant when a P value was less than 0.05.

Results

Histological analysis of knee cartilage in control group shows no changes in physiological structure. Joint surface is flat. The layered structure of the hyaline cartilage of the rats is preserved: the surface layer is represented by 5 rows of oval-elongated chondrocytes; in the transition zone, chondrocytes are rounded and are arranged singly or in isogenic groups. Most cores have clear contours and bright colors. The deep layer is represented by large hypertrophied chondrocytes (Figure 2A). There is a similar condition of cartilage tissue in PB group (Figure 2B). Histological findings of MIA-OA group shows significant disorganization of the structure of the hyaline cartilage of the knee joint. The consequence of destruction of hyaline cartilage is the replacement of fibrous connective tissue, the absence of division into superficial, intermediate and deep layers of chondrocytes. There is a reduction of the number and size of chondrocytes; replacement of the surface layer of cartilage with fibrous tissue; proliferation of fibrous and adipose tissue on the articular surface (Figure 2C). Cartilage sections of the knee joint of MIA-OA+PB group has also severe violation of the structure of the cartilage; on the other side, the cartilage morphology is damaged less comparing to MIA-OA group (Figure 2D).

The determination of the cytokine profile in the blood is an important diagnostic tool that allows assessing the functional activity of different types of immunocompetent cells, the severity of the inflammatory process at the system level and the prognosis of the course of the disease. Administration of PB has not changed the levels of ILs significantly, comparing to control group (Figure 3). MIA-induced OA caused an increasing the levels of IL-1β by 82.3%, TNF-α -81.4%, IFN-γ -55.0% and it decreased the levels of IL-4 and IL-10 by 23.3% and 24.8%, respectively, comparing to control group (Figure 4).

In this study administration of PB to animals with MIA-OA has decreased all parameters of the inflammatory profile: IL-1β decreased by 22.6%, TNF-α -21.0%, IFN-γ -22.8%, and IL-4 increased by 49.5% IL-10 -41.5%, comparing to MIA-OA group. The level of IL-12 p 40 shows no significant differences in all groups.

A role for MMPs in the pathological destruction in diseases such as rheumatoid arthritis and OA, and the irreversible nature of the ensuing cartilage and bone damage, have

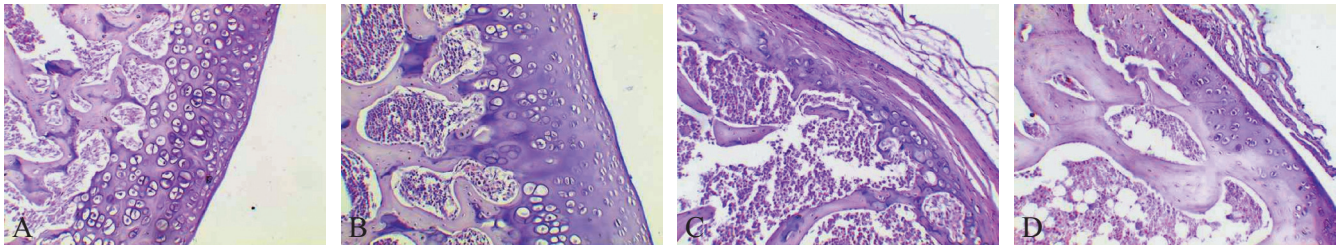

Figure 2.—Degeneration of cartilage tissue during MIA-induced OA. Light microscopic micrographs of the rat hind knee joint, GxE 200. Injections of saline to control (A) and PB (B) groups do not cause changes in cartilage surface and chondrocytes layers. Injection of MIA caused destruction of hyaline cartilage, fibrosis, reduction of chondrocytes number and intercellular matrix loss (C); the animals, which got PB course after MIA injection has similar damages, but they less common (D).

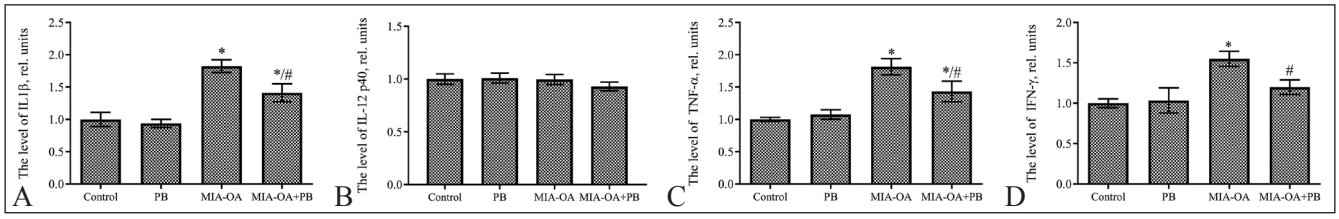

Figure 3.—The levels of pro-inflammatory CKs IL-1β (A), IL-12 p40 (B), TNF-α (C) and IFN-γ (D) in blood serum of rats, relative (rel.) units.
*P≤0.05, comparing to control group; #P≤0.05, comparing to MIA-OA group.

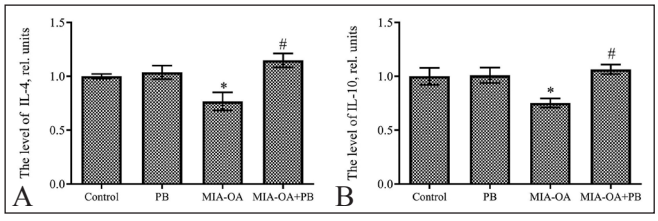

Figure 4.—The levels of anti-inflammatory CKs IL4 (A), IL-10 (B) in blood serum of rats, rel. units.
*P≤0.05, comparing to control group; #P≤0.05, comparing to MIA-OA group.

been the focus of much investigation for several decades. They also play key roles in the activity of inflammatory cells.[30] MMP1, MMP2, MMP3, MMP8 associated with the degradation and turnover of most of the components of the extracellular matrix that is why in our investigation we used them. Administration of PB has not changed the levels of MMPs significantly, comparing to control group (Figure 5). In this study, MIA-induced OA caused a significant increasing of the levels of all MMPs comparing to control group: MMP1 by 115.6%, MMP2 -101.7%, MMP3 -130.0%, MMP8 -93.4%. Administration of PB decreased the levels of MMP1 by 35.5%, MMP2 -20.6%, MMP3 -21.9% and MMP8 -28.6%, compared to MIA-OA group; these levels did not reach the values of control group.

Discussion

Current recommendations for treatment of OA include topical NSAIDs (diclofenac, capsaicin) and oral NSAIDs (paracetamol, tramadol),[31] which have high risk of side effects, including gastric and cardiovascular problems. CS widely used despite criticism of recent works about low efficiency of administration of CS during knee and hip OA in human,[32, 33] while effectivity of CS with hand OA is proven.[34] Moreover, the combination of some efficacy and low risk associated with chondroitin may explain its popularity among patients as an over-the-counter supplement.[35]

PBs may concern in similar way; they contain strains of normal intestinal microflora. Microbiome has impact on immune system and response.[36] However, the unregulated and rampant use of PBs contain a risk of turning some PB groups into opportunistic pathogens and carry the risk of plasmid-mediated antibiotic resistance transfer.[23] In our work, administration of the studying composition of PBs has not caused significant changes in the levels of CK and MMPs in blood serum and do not initiate an inflammatory response in healthy animals. The microbiome located in the human gastrointestinal tract (GIT) comprises the largest community (diverse and dense) of bacteria, and in conjunction with a conducive internal milieu, promotes the development of regulated pro- and anti-inflammatory signals within the GIT that promotes immunological and metabolic tolerance. In addition, host-microbial interactions govern GIT inflammation and provide cues for upholding metabolic regulation in both the host and microbes. Failure to regulate inflammatory responses can increase the risk of developing inflammatory conditions in the GIT.[37] Furthermore, PCR analyses of OA synovial fluid and synovial tissue have detected bacterial DNA, raising the pos-

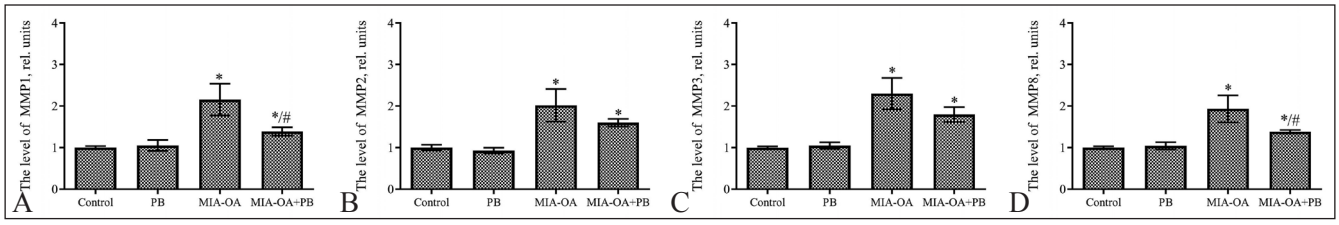

Figure 5.—The levels of MMPs MMP1 (A), MMP2 (B), MMP3 (C) and MMP8 (D) in blood serum of rats, rel. units.
*P≤0.05, comparing to control group; #P≤0.05, comparing to MIA-OA group.

sibility that live bacteria or bacterial products are present in the joint during disease progression.[38, 39]

MIA-induced OA is well-approbated model; it shows similar histopathological changes (fibrillation, fissuring, erosion, denudation, and osteophyte formation) in the joint[40] with fibrosis of cartilage surface,[41] which has similar characteristic with human OA. It is a simple method of local injection, which inhibits glycolysis and causes disruption of chondrocyte metabolism.[42] It also caused inflammatory infiltrate of monocytes, neutrophils and basophils in the joint.[43] In our study, it causes up-regulating of pro-inflammatory cytokines and MMPs and downregulating of anti-inflammatory CKs in blood serum. Moreover, it upregulated neuropeptides and microglia which leads to progressive, chronic neuronal damage that may cause neuropathic pain (hip OA sample).[44]

Previously, it was shown beneficial effect of PB feeding under OA conditions in animal models. *L. casei* suppressed experimental arthritis by down regulating Th1 effector function, which is accompanied by increased IL-10 expression.[18] Later, this study was expanding and include downregulating of IL-1β, TNF-α, MMP1, MMP3.[20] Clinical studies of *Lactobacillus* showing efficacy for treatment various pathologies, *e.g.* kidney disease, mastitis, immunomodulatory activity, gastrointestinal pathologies, insulin resistance and arthritis.[45, 46] Multi-strain probiotics showed advantages comparing with mono-strain feeding during obesity model in rats; multistrain probiotics showed ability to formed mutualistic interactions in mixtures and therefore able to share with different metabolites, affect different receptors and produced various of biologically active compounds which synergistic overall effect greater than the sum of the single effects.[47, 48] It shows perspective results in regulation of serum inflammatory cytokines during non-alcoholic fatty liver disease.[49, 50] In this study, we showed beneficial effect of the PB on the levels of pro- and anti-inflammatory CKs and MMPs in blood serum during MIA-OA in rats.

Conclusions

In this study, we have shown that an administration of the PB decreases elevated levels of pro-inflammatory CKs and MMPs in blood serum of rats with MIA-induced OA, but they have not reached the values of group without OA. The histologic assessment shows severe structural changes in knee cartilage with experimental OA including after the PB. However, the use of PBs may be perspective to improve the standard treatment of OA and it needs further investigation.

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