β-glucan-enriched fraction from mosaic puffball induces inflammation in an in vitro 3D bovine chondrocytes model

Predrag Petrović[1,*], Eda Çiftçi[2], Zhen Li[2] and Sibylle Grad[2]

[1]*Innovation Centre of the Faculty of Technology and Metallurgy, Belgrade, Serbia*
[2]*AO Research Institute, Davos, Switzerland*

Keywords: Mushrooms; Bovistella utriformis; osteoarthritis; cartilage

Hem. Ind. **78(1S)** 15 (2024)

Available on-line at the Journal web address: http://www.ache.org.rs/HI/

INTRODUCTION: Fungal β-glucans are well-known for their immunomodulatory activity. They act as pathogen-associated molecular patterns (PAMPs) and can bind to a number of pattern-recognition receptors (PRRs). Activation of PRRs leads to inflammatory response and, although these receptors are primarily found in immune cells, chondrocytes express certain types of PRRs as well (toll-like receptors –TLRs). Although β-glucans are primarily considered immune-stimulatory agents, recent research found that they may have beneficial effects in some inflammatory conditions (hence the term "immunomodulators"), in a complex way that is yet to be uncovered. The aim of this study was to investigate if the mushroom β-glucans could induce any changes in metabolic activity and phenotype of bovine chondrocytes, using a 3D cell culture model. For this purpose, glucan-enriched extract of mosaic puffball fruiting bodies, containing up to 70% (1→6)(1→3)β-D-glucan-protein complex was used.

EXPERIMENTAL: Bovine chondrocyte pellets were incubated with the extract at a concentration of 100 µg/mL for 7 days, with regular medium changes. During incubation, nitric oxide (NO) and glycosaminoglycans (GAGs) concentrations were monitored in the medium, using photometric assays [1]. At the end of the incubation, GAGs and total DNA content were determined in the pellets [1]. The gene expression of aggrecan (ACAN), collagen type 1 (COL1), collagen type 2 (COL2), interleukin-6 (IL-6), matrix metallopeptidase 3 (MMP3) and matrix metallopeptidase 13 (MMP13) was determined by real-time RT-PCR. All measurements were done in triplicate and one-way ANOVA was used for statistical analysis of the results.

RESULTS AND DISCUSSION: The treatment of the pellets with the extract caused an initial increase in the release of GAGs (by 157% on the 2nd day, compared to the control), which was followed by a steady decline and a decrease of GAG content both in the medium (by 55 %) and in the pellets (by 82 %), compared to the control at the end of the incubation period. This is consistent with the 80% decrease in ACAN expression compared to the control. The expression of COL1 and COL2 also decreased by 71 and 93 %, respectively, indicating a strong suppression of both aggrecan and collagen synthesis. The production of matrix-degrading metalloproteinases was on the other hand greatly enhanced, as expression of MMP3 and MMP13 increased by 248-fold and 217-fold, compared to the control, respectively. The IL-6 expression was also upregulated (increase by 1718-foldcompared to the control). Initially, NO production was enhanced (by 17-fold on the 2nd day of the incubation) but declined during the incubation and was 73 % lower than that in the control on the 7th day. There was no significant difference in total DNA content between the control and treated pellets, indicating that the treatment did not cause cytotoxicity.

CONCLUSIONS: In a 3D chondrocyte monoculture, the β-glucan-enriched mosaic puffball fraction exhibited strong pro-inflammatory and catabolic activity during a short-term treatment. Further research in more physiologically relevant models is needed to better understand the way β-glucan products could affect cartilage homeostasis, as well as to assess the influence of their structure, concentration, and route of administration on their potential activity.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952033.

REFERENCES

[1] Li, K., Zhu, Y., Zhang, P., Alini, M., Grad, S., & Li, Z. (2023). Anti-inflammatory and pro-anabolic effects of 5-aminosalicylic acid on human inflammatory osteoarthritis models. *J Orthop Translat*. 2023; 38: 106-116. https://doi.org/10.1016/j.jot.2022.10.003

*Corresponding author E-mail: ppetrovic@tmf.bg.ac.rs