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Proceeding Paper

Bifidobacterium animalis and *Laetiporus sulphureus* Extract Induce a Strong Increase in GSH Levels in MRC-5 Cells in Response to Oxidative Stress †

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Abstract: GSH (glutathione) is crucial for the removal and detoxification of carcinogens in healthy cells, while in cancer cells, GSH is associated with cancer expansion and increased resistance to drugs. $O_2^{\bullet-}$ acts as a secondary messenger and plays a major role in the cell signalling pathways of normal and cancer cells. Herein, the levels of $O_2^{\bullet-}$ and GSH were measured in MRC-5 and HCT-116 cells after incubation with BAL (*Bifidobacterium animalis* spp. *lactis*) and BAL/EALS (ethyl acetate extract of *Laetiporus sulphureus*) in co-culture systems, and for the first time, sensitivity was compared between these cell lines. The $O_2^{\bullet-}$ and GSH parameters were measured spectrophotometrically after 12 and 24 h. The levels of the $O_2^{\bullet-}$ were slightly increased in the MRC-5 cells after the effect of BAL and BAL/EALS (10 µg/mL), while the highest concentration of $O_2^{\bullet-}$ was recorded in treatment with BAL/EALS (50 µg/mL). On the other hand, the GSH values were elevated already after 12 h of incubation, and then further increased after 24 h in the MRC-5 cells. In the HCT-116 cells, the concentration of $O_2^{\bullet-}$ was not enhanced at 12 and 24 h of incubation compared to that of the control. The GSH level also remained relatively low. We observed a positive dose-dependent effect on the GSH levels in the MRC-5 and a negative dose-dependent effect in the HCT-116 cells. Generally, high GSH levels in the MRC-5 after 12 and 24 h indicate a strong reaction to oxidative stress and more sensitivity compared with the HCT-116 cells, where GSH stayed at a low concentration.

Keywords: healthy lung fibroblast; redox status levels; probiotics; CRC; edible mushrooms

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1. Introduction

Oxidative stress might induce genome instability and change the proliferation of healthy cells, resulting in cancer [1]. Oxidative stress can be caused by accumulated superoxide anion radicals ($O_2^{\bullet-}$), whose reactive nature is due to the presence of extra unpaired electrons. Compared to healthy cells, cancer cells have aberrant levels of $O_2^{\bullet-}$. These cells have a higher $O_2^{\bullet-}$ set point than normal cells do, which supports their growth, proliferation, metastasis, and survival. However, low or extreme levels of $O_2^{\bullet-}$ lead to instability and cancer suppression, which is the main mechanism of conventional anticancer drugs [2,3]. One of the main defence mechanisms in normal cells against $O_2^{\bullet-}$ and reactive oxygen species (ROS), in general, is glutathione (GSH). GSH neutralizes ROS in several ways, by being included in the regeneration of enzymatic and non-enzymatic antioxidants or by direct neutralization. Although in healthy cells it is crucial for the regulation of oxidative stress, elevated GSH levels in cancer cells are usually associated with their progression, as well as increased resistance to treatment [2].

In this study, for the first time, we examined the levels of $O_2^{\bullet -}$ and GSH in MRC-5 and HCT-116 cells and compared their sensitivity after incubation with BAL and BAL/EALS treatments in co-culture systems.

2. Materials and Methods

The probiotic species *Bifidobacterium animalis* spp. *lactis* (strain BB-12) (BAL) was obtained in the Microbiology Laboratory, Institute for Information Technologies, University of Kragujevac, Serbia. The detailed preparation of the BAL suspension has been described by Muruzović et al. [4].

Colorectal cancer cells (HCT-116) and healthy human lung fibroblast cells (MRC-5) were obtained from ATCC (Manassas, VA, USA). The cell lines were cultured in standard Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS) and antibiotics (100 U penicillin and 100 U/mL streptomycin).

The modified co-culture system was formed in 50 mL test tubes. A total of 40 µL of BAL diluted suspension was inoculated into 40 mL of sterile Mueller–Hinton soft agar (0.7%, *w*/*v*). Detailed instructions have been described in a study by Arsenijevic et al. [5].

Laetiporus sulphureus was gathered from the Šumadija area, Serbia (43°54′00.32″ N, 20°52′02.90″ E, Adžine Livade; altitude: 629 m). The identification and classification of the mushroom were performed with standard keys from the Mycological society "Šumadija" (Kragujevac, Serbia). Ethyl acetate solvent was used for extraction [5,6]. Ethyl acetate extract of *L. sulphureus* (EALS) was applied in two concentrations, 10 and 50 µg/mL.

GSH (reduced form of glutathione) was assessed by measuring the oxidation of the reduced form of GSH using sulfuric reagent DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)). The levels of the $O_2^{\bullet -}$ were measured by an NBT assay. The NBT assay is based on the reduction of nitro-blue tetrazolium to nitro-blue formazan in the presence of $O_2^{\bullet -}$ [7]. The $O_2^{\bullet -}$ and GSH levels were measured spectrophotometrically after 12 and 24 h.

For statistical analysis, ANOVA (SPSS for Windows, version 17, 2008, Chicago, IL, USA) was used. A statistically significant difference was $p < 0.05$ *.

3. Results and Discussion

We detected a slightly elevated level of $O_2^{\bullet -}$ in the MRC-5 cells after incubation with the BAL and BAL/EALS (10 µg/mL) treatments, while a significant increase in $O_2^{\bullet -}$ was only observed in the treatment with BAL/EALS (50 µg/mL) (Figure 1A). However, the concentration of GSH was significantly elevated in all the treatments compared to that of the control. We noticed the positive dose-dependent effects of the treatments on the GSH parameters in the MRC-5 cell line (Figure 1B). When it comes to the HCT-116 cells, the $O_2^{\bullet -}$ levels remained almost unchanged, while the treatments induced a dose-dependent decrease in GSH (Figure 1C, D). Increased concentrations of GSH in the MRC-5 cells indicate the occurrence of oxidative stress and greater sensitivity of these cells to treatment. On the other hand, the HCT-116 cells showed greater resistance to the tested treatments, which can be concluded based on relatively low values of $O_2^{\bullet -}$ and GSH compared to those of the MRC-5 cells.

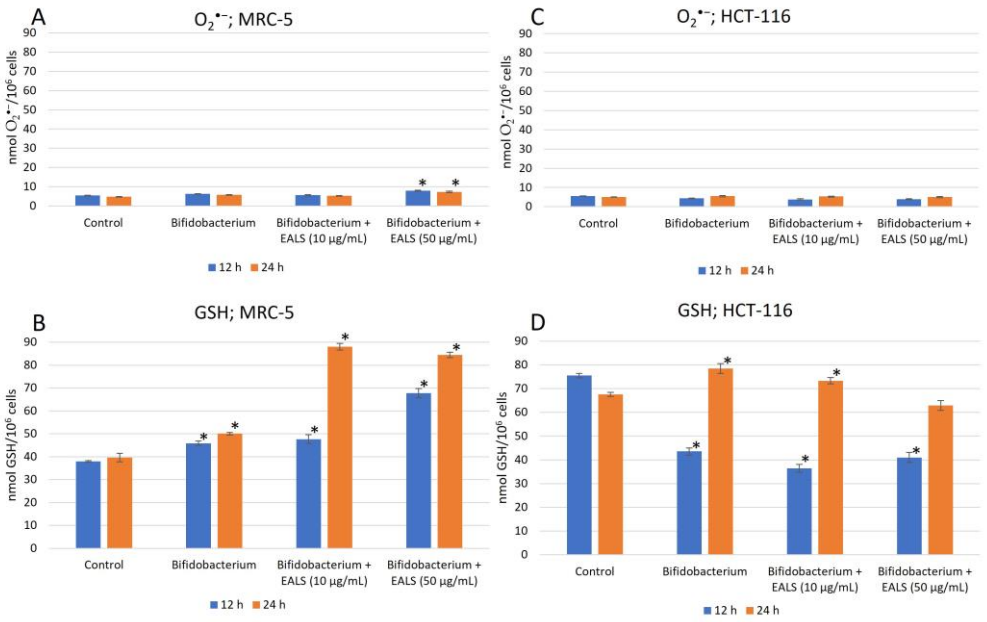

Figure 1. Levels of $O_2^{\bullet-}$ and GSH in MRC-5 (**A**,**B**) and HCT-116 cells (**C**,**D**) after the treatment with BAL and BAL/EALS (10 and 50 μg/mL). * Statistical significance shows a difference between the control and treatments at 12 and 24 h of incubation.

4. Conclusions

The results of our study indicate the strong sensitivity of MRC-5 cells to the applied treatments compared to that of the HCT-116 cells that show resistance. This can be concluded from the high GSH values in the MRC-5 cells that activated the defence mechanism against oxidative stress.

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