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ACTION OF LOW MOLECULAR WEIGHT GLUCAN ON TRASCRIPTOME OF NEOPLASTIC CELL LINES

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Abstract – *Objective:* *Glucans are already known as biological modifiers of the immune system and cytokine modulation (IFN-y; TNF; T-CD4/CD8), but the role of low molecular weight glucans on transcriptome regulation is not well documented.*

Materials and Methods: *In this paper, the mechanism of action of a mix in capsules of 0.6 g of β-Glucan (Active hexose correlated compound "AHCC" and β-Glucan 1,3-1,6) on the neoplastic cell line transcriptome has been explored. The cellular models used were Caco2 and LS174T cell lines. Transcriptome analysis was performed by RNA expression analysis of transcripts of both single and multiple samples. The analysis was completed with the aid of information technology.*

Results: *ROS (Reactive Oxygen Species) decrease following glucans treatment (Figure 1) combined with a marked improvement in the Redox control of SOD1 (Superoxide Dismutase 1) (Figure 2), which is pre-sent in the cytosol of all eukaryotic cells with copper and zinc (Cu-Zn-SOD). Following glucan mix treatment, COX-2 (cyclooxygenase-2) expression levels showed a marked reduction (Figure 3A). The combined glucan treatment on the two cell lines showed an over expression of SOD1 with a significant reduction in COX-2 expression (Figure 3A). Pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, were consequently down-expressed (Figure 3B, C, D).*

Conclusions: *The glucan mix reduces ROS significantly, decreasing COX-2 expression, reducing the inflammatory state and consequently decreasing TNF-α and IL-1.*

KEYWORDS: *Beta-glucan, Inflammation, Cancer.*

INTRODUCTION

An increasing number of phytocomplexes have been studied to modulate and stimulate the immune response in immunocompromised subjects[1-4]. Low molecular weight glucans are among the most known to be biological response modifiers (BRMs) that stimulate the immune system through the activation of different immune cells including macrophages, dendritic cells, neutrophils, natural killer (NK) and lymphocytes. BRMs are used for cancer therapy in association with cytotoxic-chemotherapeutic agents: several authors describe the improvement of the immunological response *in vivo* in subjects suffering from cancer[5-7]. The transcriptome after receptor stimulation of the polysaccharides AHCC + β Glucan of the *Lentinula Edodes* and *Saccharomyces Cerevisiae* is little studied, although those latter are commercially present as dietary supplements, orally bioavailable, well bear

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by humans and without adverse effects[8-13]. Chemical analysis has shown that AHCC is made up of about 54% from oligosaccharides containing about 40% of the α-1,4-glucan type with low molecular weight (5 kDa) and the remaining part is β-Glucan (1,3-1,6). From the literature we know that the effects of these polysaccharides are more evident in subjects with impaired immune function[14-28]. In this research we performed ROS and transcriptome analysis in Caco2 and LS174T cell lines to understand the improvement of oxidative and pro-inflammatory state following glucan treatment.

MATERIALS AND METHODS

Cell cultures

LS174T and Caco-2 cells were obtained from the ATCC (American Type Culture Collection, Manassas, VA, USA). LS174T cells were grown in Advanced modified Eagle medium (A-MEM, Gibco, Rockville, MD, USA) while Caco-2 cells were grown in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, St. Louis, MO, USA).

Experimental conditions

Experiments were performed according to the conditions described in Table 1.

Quantitative reverse transcription-PCR (real-time RT-PCR)

The mRNA level of SOD1, COX-2, TNF-α, IL-1, IL-6, was determined by quantitative real-time polymerase chain reaction (q-RT-PCR). To measure gene expression, total RNA from cultured cells was extracted using RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA concentration and purity were checked using Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). qRT-PCR was performed for each gene of interest using a 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) together with specific primers. Data were normalized to the 18S as the housekeeping gene. All experiments were performed at least three times and analysis was performed in duplicate for each experiment. The delta-delta cycle-threshold (ΔΔCt) method was used to analyze the fold change in mRNA expression from qRT-PCR experiments.

Intracellular ROS detection assay

Cells were washed with 100 µl of Krebs-HEPES buffer (10 mM HEPES, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 11 mM D-(+)-glucose and pH 7.4) and incubated for 45 min with 10 µM H2DCF-DA at 37°C and 5% of CO_2. The dye was removed and replaced with Krebs-HEPES buffer. The fluorescence intensity was measured using Ensight Multimode Plate Reader at excitation/emission = 485/535 nm and used to calculate the fold change.

Statistical analysis

Data were normalized and shown as the mean ± standard error of the mean (SEM) of three independent experiments performed. A One-Way Analysis of Variance was used to calculate statistical significance of normalized data, followed by least significant difference analysis. Results were considered significant when the probability was <0.05 ($p < 0.05$) and 0.01 ($p < 0.01$).

RESULTS

ROS decrease following glucans treatment (Figure 1) combined with a marked improvement in the Redox control of SOD1 (Figure 2), which is present in the cytosol of all eukaryotic cells with copper and zinc (Cu-Zn-SOD). Superoxide is one of the major oxidizing agents in the cell and consequently, SOD has an antioxidant key role[29-31]. The physiological

TABLE 1. Experimental conditions.

Caco-2 ctr (control)	LS174T ctr (control)
Caco-2 AHCC 100 µM	LS174T AHCC 100 µM
Caco-2 β-Glucan 100 µM	LS174T β-Glucan 100 µM
Caco-2 Vitamin C 25 µM	LS174T Vitamin C 25 µM
Caco-2 AHCC 100 µM + β-Glucan 100 µM	LS174T AHCC 100 µM + β-Glucan 100 µM
Caco-2 AHCC 100 µM + β-Glucan 100 µM + Vitamin C 25 µM	LS174T AHCC 100 µM + β-Glucan 100 µM + Vitamin C 25 µM

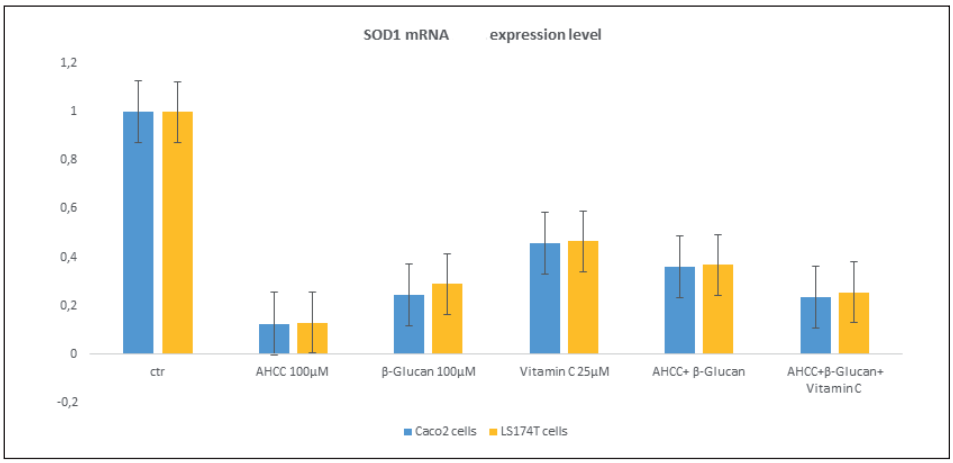

Fig. 1. SOD1 mRNA expression level determined by RT-qPCR. Results are means of 3 independent experiments ± SD, $p<0.05$.

importance of SODs is evident in serious pathologies in mice genetically missing of these enzymes. Mice lacking SOD2 die a few days after birth due to strong oxidative stress. Those lacking SOD1 develop a wide variety of diseases, including hepatocellular carcinoma, an acceleration of age-related loss of muscle mass, familial amyotrophic lateral sclerosis (ALS, a form of motor neuron disease).

Following glucan mix treatment, COX-2 expression levels showed a marked reduction (Figure 3A).

COX-2 is the enzyme that converts arachidonic acid into prostanoids: thromboxanes and prostaglandins; it is an over-expressed inducible enzyme at the site of inflammation[32-38]. The COX-2 overexpression determines resistance to apoptotic phenomena, blocks the P53 protein, over-expresses the anti-apoptotic protein Bcl-2 and reduces the expression of the pro-apoptotic protein Bax. Chemotherapeutic and radiotherapeutic agents enhance the expression of the COX-2 enzyme in human cancer cells and this leads to resistance to therapy.

The combined glucan treatment on the two cell lines showed an over expression of SOD1 with a significant reduction in COX-2 expression (Figure 3A). Pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, were consequently down-expressed (Figure 3B, C, D).

DISCUSSION

The values obtained indicate an inflammatory state secondary to a host immune response following a biotic and/or abiotic stimulus recognized as no-self. In summary, glucan treatment produced a better control of cellular homeostasis and inflammation. Chemotherapeutic and radiotherapeutic agents normally promote the over-expression of COX-2; therefore, the management with the glucan mix could favor a better response to cancer treatments.

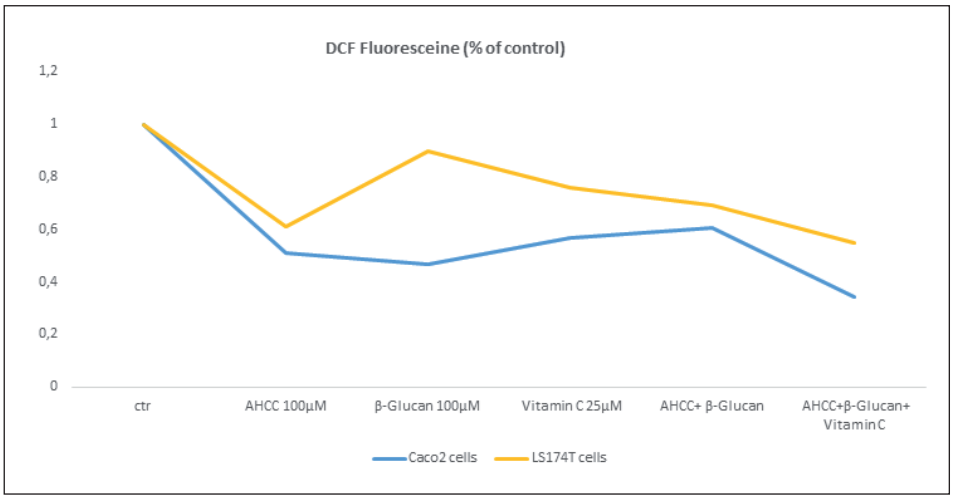

Fig. 2. ROS generation following treatment of Caco2 and LS174T with glucans over a 4 h time course. Fluorescence intensity Results are expressed as mean ± SEM from four independent experiments and statistical significance was determined by One-way ANOVA followed by least significant difference post-hoc analysis. $p<0.01$ compared with untreated control.

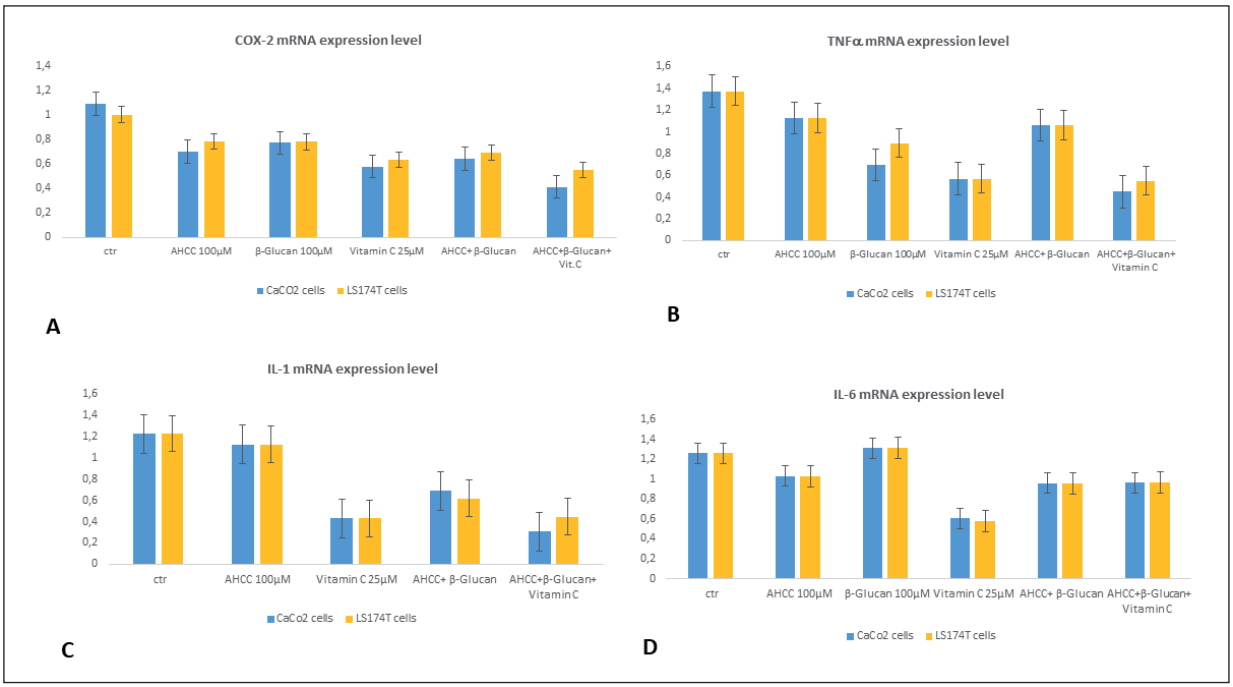

Fig. 3. Expression level of COX-2, IL-1, TNF-α, and IL-6 mRNA were determined by RT-qPCR. Results are means of 3 independent experiments ± SD, *$p<0.05$.

CONCLUSIONS

The production of cellular ROS was high in the absence of treatment with glucans, while it was decreased after treatment with combined glucans mix. The lowering of ROS reduced the activity of COX-2, which is induced by an oxidative and pro-inflammatory state, and consequently the expression of TNF-α and IL-1 was decreased.

In conclusion, glucan mix reduces ROS significantly, decreasing COX-2 expression, reducing the inflammatory state and consequently decreasing TNF-α and IL-1.

Conflict of Interest:

The Authors declare that they have no conflict of interests.

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