

Chlorophyllin changes the expression of apoptosis and cell cycle genes in non-tumor breast HB4a cells

Gláucia Fernanda Rocha D'Epiro^{1*}; Simone Cristine Semprebon¹; Andressa Megumi Niwa¹; Lilian Areal Marques¹; Mário Sérgio Mantovani¹

¹ Universidade Estadual de Londrina, Londrina –PR.
*glauucia_depairo@hotmail.com

Introduction: The molecule of chlorophyllin has been the subject of the scientific community by presenting chemopreventive, antimutagenic and anticarcinogenic properties. However, the molecular mechanisms of action of this compound remain unclear. Thus, the study of genes involved in apoptosis and cell cycle in non-tumor cells treated with chlorophyllin can provide important information about the mechanisms that protect or trigger damaging processes, aiding in the development of therapies. **Objective:** In the present study was evaluated the cytotoxicity of chlorophyllin and its effect on expression of genes involved in apoptosis (anti and pro-apoptotic: *BCL-2*, *BCL-XL*, *BAX* and *BAK*, Caspases: *CASP3*, *CASP7*, *CASP8* and *CASP9*) and cell cycle (*BIRC5*, *TP53*, *APC*, β -*catenin* and *CCNA2*) on cultured non-tumor breast cells (HB4a). **Methods:** The cytotoxic potential of chlorophyllin was investigated using the MTT assay at 100, 200 e 400 $\mu\text{g/mL}$ of chlorophyllin, for 24, 48 and 72 hours. The study of gene expression was performed by qRT-PCR assay at 100 and 200 $\mu\text{g/mL}$ of chlorophyllin for 24, 48 and 72 h. **Results:** Chlorophyllin was cytotoxic at 48 and 72 h at all concentrations (100, 200 and 400 $\mu\text{g/mL}$) when compared to control. The reduced levels of *BIRC5* related to the processes of apoptosis and cell cycle was dose dependent, the gene was downregulated with 200 $\mu\text{g/mL}$ of chlorophyllin at 24, 48 and 72 h. The *CCNA2* gene was downregulated at 24, 48 and 72 h with 200 $\mu\text{g/mL}$ of chlorophyllin, and at 72 h with 100 $\mu\text{g/mL}$ of exposure. In relation to the pro-apoptotic genes, it was observed a significant increase in expression of *BAX* at 24, 48 and 72 h of exposure at 200 $\mu\text{g/mL}$ of chlorophyllin, and 72 h of exposure at 100 $\mu\text{g/mL}$. It was also observed increased expression of anti-apoptotic *BCL-2* and *BXL-XL*. The gene *BCL-XL* showed increased expression in three times tested with 200 $\mu\text{g/mL}$ of chlorophyllin, and exposure to 100 $\mu\text{g/mL}$ led to increased expression in 24 h. Since the *BCL-2* gene had an increase of expression only in 72 hours with 200 $\mu\text{g/mL}$ of chlorophyllin. The other genes analyzed did not show significant change. **Conclusion:** Inhibition on genes expression involved in cell cycle suggests an effect due to the cytotoxicity of chlorophyllin, while the increased expression of anti-apoptotic genes suggests inhibiting apoptosis and protection from death cellular induced by chlorophyllin.

Keywords: chlorophyllin, cell cycle, apoptosis, gene expression.

Financial support: CNPq, CAPES and Fundação Araucária