Effect of Fusarium Mycotoxin Zearalenone on Gut Epithelium

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Bulletin UASVM Animal Science and Biotechnologies 71(2) / 2014,

Print ISSN 1843-5262; Electronic ISSN 1843-536X

DOI: 10.15835/buasvmcn-asb:10543

Abstract

In the present study, *in vitro* experiments were conducted to evaluate the changes produced by fusariotoxin, Zearalenone (ZEA), on global gene expression in human intestinal epithelial cells (Caco2), knowing that the intestine is the first organ exposed and affected by the mycotoxin ingestion during an oral intoxication. Caco2 cells were treated with 10µM of ZEA for 24 hours and the changes in global genes expression was investigated by microarray and analysed by using Gene Spring GX v.11.5 software. Our results showed that ZEA at the concentration of 10µM did not affect the cell viability but, the microarray analyses revealed that this mycotoxin was able to induced significant changes in genes expression profile in Caco2 cells in comparison with control cells. The up-regulation gene expression was the most common effect produced by ZEA in Caco2 cells after 24h of exposure to 10µM of ZEA. Microarray analyses identified 377 genes differentially regulated of which 11 genes were down-regulated and 319 up-regulated with a fold change ≥ 2 . ZEA was also able to increase the expression of certain cytokines (IL-1 β , IL-6, IL-8 and IFN- γ) involved in inflammation, signalling molecules (ERK1, ERK2, JNK1, JNK2, p38- α) and nuclear receptors (NF-kB and PPAR- γ), which control the immune functions. Together all these results suggest that zearalenone is a genotoxic mycotoxin able to induced alterations in a significant number of genes involved in different molecular and functional processes at intestinal level.

Keywords: zearalenone, gene expression, microarray, human intestinal Caco-2 cells

Introduction. Zearalenone is a mycotoxin produced by *Fusarium sp.*, very known for its oestrogenic properties (Tabuc *et al.*, 2009). In addition, ZEA has been shown to be immunotoxic and hepatonephrotoxic, which means a greater risk factor for public health (Tiemann and Dänicke, 2007; Kuiper *et al.*, 1998). Although the studies on ZEA intensified in the last period, the mechanisms of its toxicity need to be evaluated. Considering that the intestine represents the main route of intoxication with mycotoxins (Pinton *et al.*, 2010) a microarray approach could generate important genomic overview necessary to elucidate the interaction of this toxin with the genes involved in different metabolic processes (proliferation

and cell differentiation, apoptosis, inflammation, protein synthesis etc) at intestinal level.

Aims and objectives. In the present study, *in vitro* experiments were conducted to evaluate the changes produced by ZEA on global gene expression of epithelial intestinal cells taking into consideration that the intestine is the first organ exposed and affected by the mycotoxin ingestion during an oral intoxication.

Materials and methods. Caco-2 cells were seeded at 2.0×10^5 cells concentration in 24-well cell culture plates (Costar, Corning, NY, USA) until they reached confluence (2-3 days). Cells were treated were with 10µM of pure ZEA dissolved in ethanol/culture media (1:1, v:v) for

Fig. 1a. Microarray down-regulated genes.

Fig. 1b. Microarray up-regulated genes

24 hours. Changes in genes expression profile was

investigated by microarray and qPCR using Agilent technology and methodology. Agilent Gene Spring GX v.11.5 software was used to analyze expression profile of altered genes. Genes with fold change ≥ 2 and a P value < 0.05 were considered differentially expressed.

Results and discussion. Our results showed that ZEA at the concentration of $10\mu\text{M}$ did not affect the cell viability but the microarray analyses revealed that this mycotoxin was able to induced significant changes in genes expression profile in Caco2 cells in comparison with control cells. The up-regulation gene expression was the most common effect produced by ZEA in Caco2 cells after 24h of exposure to $10\mu\text{M}$ of ZEA. Thus, Microarray analyses identified 377 genes differentially regulated of which 11 genes were

down-regulated and 319 up-regulated with a fold change ≥ 2 (Fig.1a and Fig.1b). 102 of up-regulated genes had a fold change between 3 and 6. The highest expressed gene (19.26 fold change) was STRN3 gene encoding for calmodulin protein, which mediate many crucial processes such as inflammation, metabolism, apoptosis etc.

ZEA was also able to increase the expression of other marker of inflammation like cytokines (IL- 1β , IL-6, IL-8 and IFN- γ), signaling molecules (ERK1, ERK2, JNK1, JNK2, p38- α) and nuclear receptors (NF-kB and PPAR- γ), which control the immune functions.

Conclusion. Together all these results suggest that zearalenone is a mycotoxin, which produce genotoxic effect able to induced alterations in a significant number of genes involved in different molecular and functional processes at intestinal level.

Acknowledgements. This work was supported by funds from the National Research Project PNII-ID PCE 2011-3-0339-101/2011-2015 granted by the Romanian Ministry of Research and Technology.

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