Mycotoxin Zearalenone induced apoptosis in BEAS-2B cells through generation of ROS and activation of JNK and p38 MAPKs signaling pathway

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Abstract

Human exposure to Zearalenone (ZEA, a non-steroidal estrogenic mycotoxin) through inhalation has raised considerable concern. However, the potential health risk and the mechanism of actions of ZEA are not well understood. In the present study, we used BEAS-2B, cultured human bronchial epithelial cells, as well as Cygb (a free radical scavenger) over-expressing BEAS-2B cells to study the cytotoxic effects of ZEA. Our results indicated that ZEA decreased cell viability, induced apoptosis and promoted ROS formation in BEAS-2B cells. Oxidative stress was clearly evident, as shown by elevated mRNA expression levels of oxidative stress markers (Hsp70 and Hsp27) and endogenous antioxidants (SOD2 and Gpx). Over expression of Cygb reduced level of reactive oxygen species (ROS) and the percentage of apoptotic cells induced by ZEA. Cells pre-treated with either p38 or JNK inhibitors showed no attenuation in ROS levels, but the percentage of apoptotic cells was lower than cells treated with ZEA alone. Overall, our results indicated that ZEA induces apoptosis, possibly through generation of ROS and activation of JNK and/or p38 MAPK signaling pathways.

Introduction & Objectives

ZEA is a mycotoxin found in contaminated grain, maize and barley. There are also detectable levels of ZEA in the air, however there is very limited data on its effects on the lungs. Epithelial cells are the first line of exposure upon inhalation of ZEA. BEAS-2B cells, which is derived from human lung epithelium, are used here to study the cytotoxicity and cell-death pathways following exposure to ZEA. Cytoglobin is a ubiquitously expressed protein well known for providing protective effects against oxidative stress by acting as a free radical scavenger [2]. To study the correlation between oxidative stress and ZEA-induced cytotoxicity, BEAS-2B cell line over-expressing Cygb were also used.

Methods

Wild type (Wt) and Cygb transfected (Cy) BEAS-2B cells

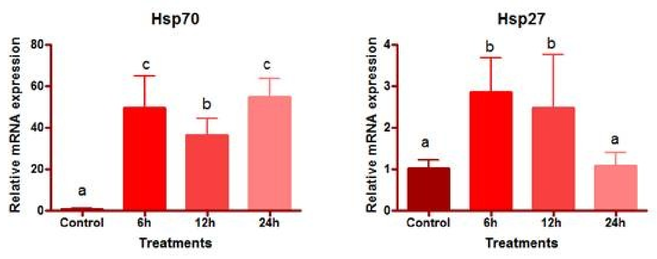
0.05% DMSO Control/ 40uM ZEA treatment (the LOAEL* for both cell lines after 48h treatment, determined from MTT assay)

- Extent of ROS generation → DCFH-DA assay (flow cytomtery)
- Levels of oxidative stress related genes → RNA extraction, RT-PCR, Real time PCR
- Apoptotic status of cells → Annexin V/PI staining (flow cytomtery)

*LOAEL=Lowest Observable Adverse Effect Level
All assays have n=3 and were did in triplicate

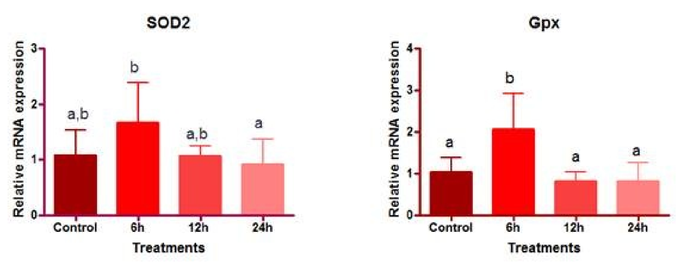
Results & Discussions

ZEA ↑ oxidative stress markers

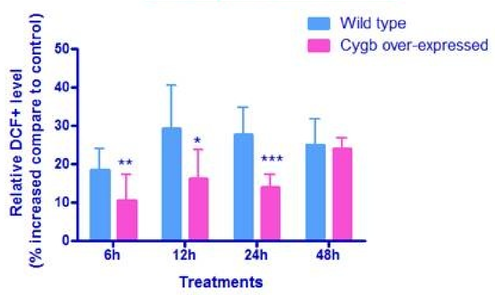


40μM ZEA induced the expression of oxidative stress related genes including heat Shock protein 70 (Hsp70), heat shock protein 27 (Hsp27), glutathione peroxidase (Gpx) and superoxide dismutase 2 (SOD2) in wild type cells. Data are expressed as mean ± SEM . Bars with different alphabet represent $p<0.05$ significant different.

ZEA ↑ endogenous antioxidants

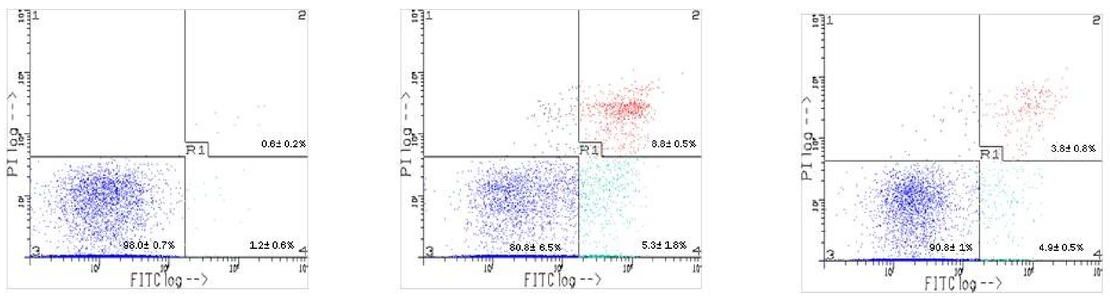


ZEA ↑ ROS levels

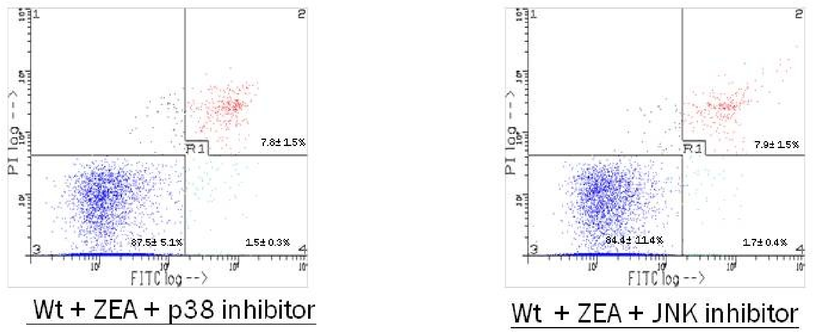


40μM ZEA induced ROS generation in both cell lines Over-expression of Cygb significantly reduced ROS level. Data are expressed as mean±SD. *, ** and *** represent $p<0.001$, $p<0.01$ and $p<0.05$ signifcantly different from respective wild type values.

ZEA induced apoptosis



Control | Wt treated with ZEA | Cy treated with ZEA



Wt + ZEA + p38 inhibitor | Wt + ZEA + JNK inhibitor

Over-expression of Cygb or pretreatment with either p38 or JNK inhibitor significantly reduced ZEA-induced apoptotic cells. *Q2 is late apoptotic or necrotic cells, Q3 is living cells and Q4 is early apoptotic cells.

Conclusions

Our results demonstrated that ZEA induced oxidative stress and provoked apoptotic cell death in BEAS-2B cells. JNK and p38 MAPK signaling possibly play a crucial role in ZEA-induced apoptosis in BEAS-2B cells. This study provide experimental evidence showing the possible cytotoxic effects of ZEA to lung cells.

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References:

1. Burmester T et al., *Molecular biology and evolution*. **19**, 416-421 (2002)