NIVALENOLENOL AND DEOXYNIVALENOL INDUCE APOPTOSIS AND DYSREGULATE WOUND REPAIR IN RAT INTESTINAL EPITHELIAL CELLS

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Introduction

The gastrointestinal tract represents the first barrier against ingested chemicals, food contaminants and toxins thus its integrity represents a barrier between the internal and external environments. *Fusarium* mycotoxins, nivalenol (NIV) and deoxynivalenol (DON) are frequently on cereals and processed grains. Following their ingestion, intestinal epithelial cells are exposed to high concentrations of NIV and DON capable to induce mycotoxicosis (Yang et al., 2010). To investigate the effects of NIV and DON we used the intestinal epithelial cell line (IEC-6).

Methods

IEC-6 cells were culture in cultured using Dulbecco's modified Eagle's medium (4 g/L glucose) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2 mm L-glutamine, 1.5g/L $NaHCO_3$, and 0.1 unit/ml bovine insulin. Cells were used at the 17^{th}-21^{st} passage. Cell viability was assessed through MTT assay (Mosmann, 1983). The effects on apoptosis induction and cell cycle progression were analyzed using PI staining by flow cytometry. Finally, the effects on wound healing were assessed by measuring the migration rate of individual cells by time laps microscopy.

Results and Discussion

NIV and DON (0.1-2.5µM) was assessed for their ability to modulate epithelial cell migration, the initial step of gastrointestinal wound healing. The addition of NIV in the culture medium significantly reduced the rate of migration of IEC-6 cells into the denuded area of a model wound. Instead DON slightly reduced the rate of migration of IEC-6 cells into the denuded area compared to IEC-6 cells cultured alone. Interestingly a synergic activity on reducing the rate of migration was observed adding to IEC-6 cells NIV and DON mycotoxins together. Both NIV and DON, tested at higher concentrations (5-80µM) significantly affected IEC-6 cells viability. Moreover propidium iodide analysis revealed that the reduced cell viability was related to an apoptotic process. All together our results reported the effect of NIV and DON on intestinal epithelium highlighting the effect of the few studied NIV and the synergistic activity of both mycotoxins in reducing the IEC-6cell response to epithelial injury.

References

- Yang, H., et al. 2010. Mechanism-based alternative monitoring of endoplasmic reticulum stress by 8-keto-trichothecene mycotoxins using human intestinal epithelial cell line, Toxicol.Letters 198, 317-323.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65, 55-63.