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Evaluation of the cytotoxic effects induced by ochratoxin A in a bovine mammary cell line

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INTRODUCTION

- Ochratoxin A (OTA) is a metabolite produced by both *Aspergillus* and *Penicillium* species.
- OTA is a nephrotoxic, immunotoxic, cancerogenic and teratogenic compound.
- OTA mechanism of action: it can act by inducing cytotoxicity, oxidative cell damage and increased cell injury.

OBJECTIVE

The aim of the present study was to evaluate the damages induced by OTA in an in vitro model of the bovine mammary epithelium.

MATERIALS and METHODS

EXPERIMENTAL PROCEDURE

- BME-UV1 (Bovine Mammary Epithelia – University of Vermont – clone 1) cell line was cultivated in culture medium at 37°C in a humidified 5% CO_2 incubator.
- OTA was solubilised in methanol and diluted in DMEM in order to obtain the final concentrations (0-0.07-0.15-0.3-0.6-1.2-2.5 µg/ml) used in the further experiments.
- The effect of OTA treatments on cell viability after 24, 48 and 72 h was evaluated by MTT test. Then, the OTA Lethal Concentration 50 (LC_{50}) was calculated.
- The impact of OTA treatments on cell membrane damage after 24, 48 and 72 h was a assessed by measuring lactate dehydrogenase (LDH) release.
- In order to detect DNA damage induced by OTA treatments, quantitative analysis of DNA fragmentation was performed after 24 h of OTA treatment by the diphenylamine method.
- The detection of DNA fragmentation was evaluated also by TUNEL assay.
- At least three replicates per treatment were performed and the experiments were repeated twice.

STATISTICAL ANALYSIS

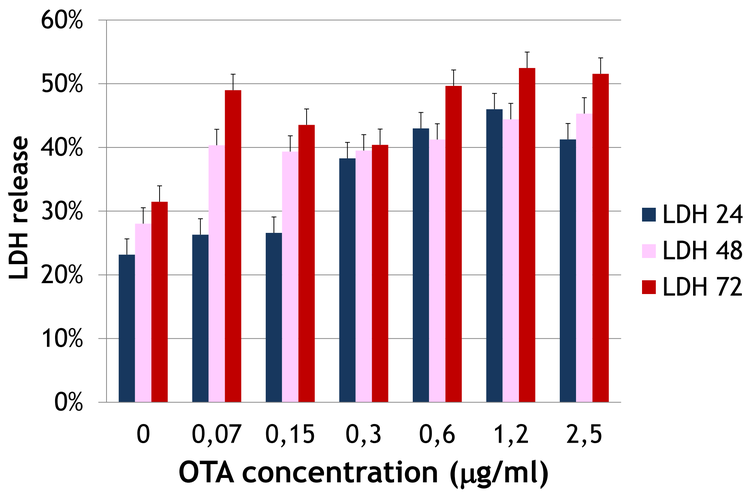
The obtained data were analysed by one-way ANOVA, with $P \leq 0.05$ considered statistically significant.

RESULTS

LC_{50} OTA (µg/ml)	24h	48h	72h
BME-UV1	0.8	<D.L.*	<D.L.*

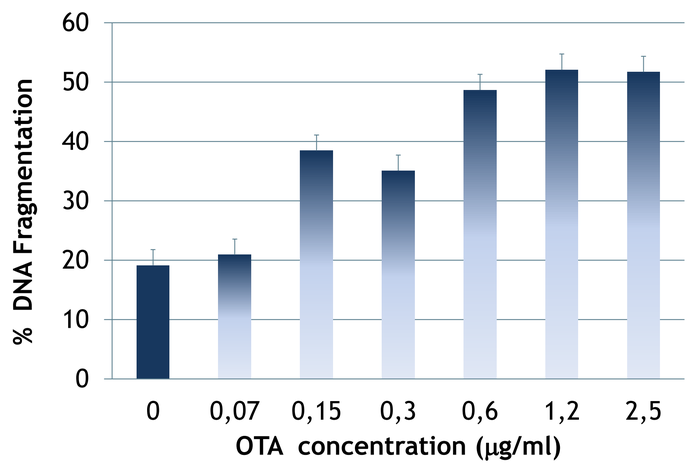
*D.L.: detectable level.

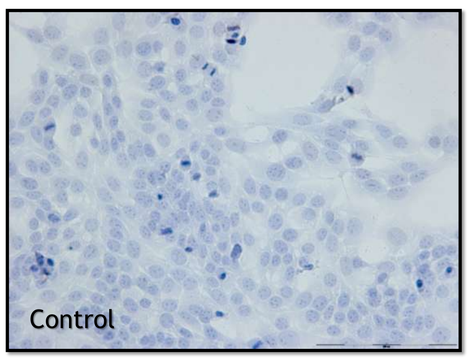
BME-UV1 cells appeared to be sensitive to OTA cytotoxicity.

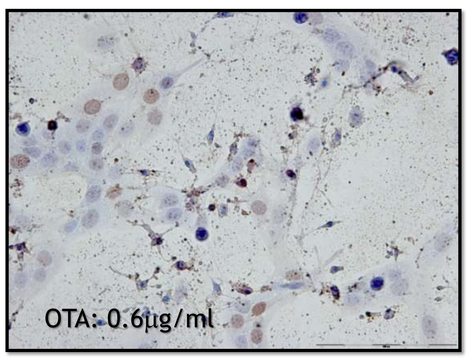


LDH release by BME-UV1 cells in the presence of several concentrations of OTA were significantly increased compared to the control.

DNA fragmentation, evaluated by the diphenylamine method, was found to be dose-dependent after 24 h of OTA treatment.







After 24h of OTA treatment (0.6 µg/ml), the monolayers were completely destroyed and cell debris invaded all microscope fields when compared to control cells (Magnification: 200x).

CONCLUSIONS

- In BME-UV1 cells OTA was able to affect cell viability and to induce cellular damage, as shown by LDH release and DNA fragmentation.
- The mechanisms by which OTA induces its toxicity depends by the concentration/dosage and the time-exposure used.