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APOPTOSIS-INDUCING EFFECT OF AFLATOXIN B1, OCHRATOXIN A AND ZEARALENONE COMBINATION IN RAT HEPATOCYTES

T. A. HARUTYUNYAN *

Chair of Genetics and Cytology YSU, Armenia

Aflatoxin B1 (AFB1), ochratoxin A (OTA) and zearalenone (ZEA) are widespread mycotoxins with carcinogenic properties, contaminating feed and food. In this study the apoptosis-inducing effect of AFB1, OTA and ZEA combination at concentrations close to the natural contamination, in rat hepatocytes after 15, 30 and 60 days treatment *in vivo* is presented.

***Keywords*:** mycotoxins combination, apoptosis, rat hepatocytes, annexin V.

Introduction. Mycotoxins are secondary metabolites of mould and ubiquitous contaminants of food and feed. In terms of toxicity the most harmful mycotoxins are aflatoxins AF (B1, B2, G1, G2), ochratoxin A (OTA) and zearalenone (ZEA), which co-occur in foods [1] and have apoptosis-inducing and hepatotoxic effects [2–6]. The combined action of these mycotoxins at concentrations close to the natural contamination is not studied in rat hepatocytes.

Apoptosis of mammalian cells is accompanied by various morphological changes including externalization of phosphatidylserine (PS) – an early hallmark of apoptotic cells [7]. One of the modern approaches for distinction of apoptotic, necrotic and viable cells in the same population is application of annexin V-Cy3 in combination with vital dye (6-carboxyfluorescein diacetate – 6-CFDA).

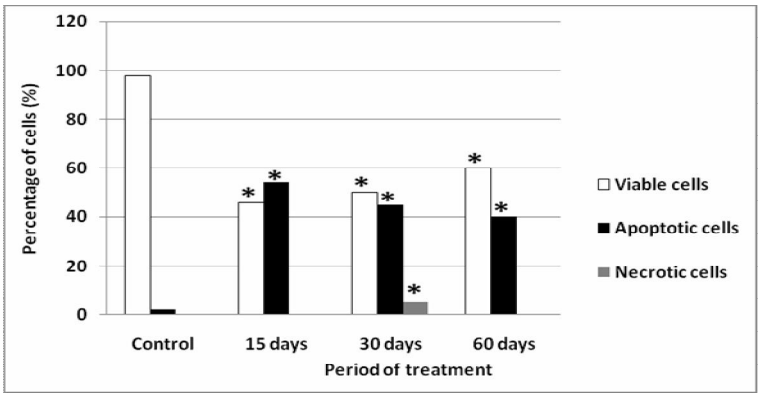
Materials and Methods. Wistar rats (200±20 *g*) were kept in standard environmental conditions and randomly assigned to four groups of 5 animals, each receiving mixture of AFB1 (0.57 $\mu g/kg$/day), OTA (0.87 $\mu g/kg$/day) and ZEA (0.95 $\mu g/kg$/day) or solvent only (control group) for 15, 30 and 60 days. The doses of mycotoxins were selected on the base of data on individual cytotoxicity [2, 4, 5] in rodents. For detection of cell death pathway liver samples were collected and hepatocytes were isolated according to Wang et al. [8]. Apoptosis detection in hepatocytes was performed by staining PS, exposed on outer leaflet of the cell with Cy3.18-labeled annexin V (red fluorescence). For membrane integrity detection the vital dye 6-CFDA (green fluorescence) was used [7]. Cells (apoptotic, necrotic and viable) were scored under fluorescence microscope (“Zeiss”, Germany) at 250×magnification. Generally, 4–6 representative fields of at least 100 cells were

* E-mail: tigr-har@mail.ru

scored for analysis. Statistical analysis of the results was performed using Chi-square online program [9].

Results and Discussion. Analysis of obtained results revealed that mixture of AFB1, OTA and ZEA significantly ($p<0.05$) increased the level of apoptosis in all treated groups (see Figure). Namely, the level of *apoptotic cells* enhanced after 15 days treatment from 2% (control) to 54%, and decreased after 30 and 60 days, remaining at significantly high levels (45% and 40% respectively) compared to control. The level of *viable cells* decreased after 15 days of treatment from 98% (control) to 46%, but increased after 30 and 60 days (50% and 60% respectively), which significantly differs from control. Necrotic cells (5%) were detected only after 30 days treatment.

Studying on apoptosis-inducing effects of mycotoxins *in vivo* mainly high doses of AFB1 (0.3 *mg*/*kg*), OTA (120 *μg*/*kg*) and ZEA (5 *mg*/*kg*) were analyzed [10–13]. Therefore, it was interesting to investigate the chronic exposure of mixture of these mycotoxins at concentrations close to the natural contamination on rats hepatocytes.



Comparison of levels of viable, apoptotic and necrotic cells in rat hepatocytes treated with mixture of AFB1, OTA and ZEA (* $p<0.05$ compared with control).

Conclusion. The obtained results suggest that chronic exposure to mixture of mycotoxins through food consumption can induce apoptosis in rat hepatocytes and decrease the level of viable cells. The double staining method with AnnCy3 and 6-CFDA demonstrated high specificity in rat hepatocytes for detection of cell death pathway *in vivo*.

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