Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

•Փորձարարական և տեսական հոդվածներ •Экспериментальные и теоретические статьи•
•Experimental and theoretical articles•

Biolog. Journal of Armenia, 2 (67), 2015

EVALUATION OF GENOTOXIC EFFECTS OF OCHRATOXIN A IN THE RAT BONE MARROW CELLS *IN VIVO*

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Ochratoxin A (OTA) is a mycotoxin that contaminates food and feed and has been classified as a possible human carcinogen. Human exposure to OTA is worldwide. Here the genotoxic potential of OTA (4.375 μg/kg body weight/day for 15, 30, and 60 days) in the bone marrow cells of adult Wistar rats exposed to mycotoxin through food consumption was described. Level of DNA damage was measured by using standard alkaline single-cell gel electrophoresis (comet assay). The tail intensity and tail moment of DNA-comets in bone marrow cells were significantly higher in the groups treated for 15 and 60 days than in controls ($p<0.05$). These parameters were also significantly higher in comparison to the group treated for 30 days ($p<0.05$). The highest tail intensity and tail moment was observed in animals treated for 15 days, and it differed significantly from animals treated for 60 days ($p<0.05$). Our results confirm the genotoxic potential of OTA and demonstrate its activity in low concentrations in rat bone marrow cells by comet assay.

Ochratoxin A – mycotoxins – genotoxicity – rat bone marrow cells – DNA comet assay

Օխրատոքսին A (OTA) միկոտոքսին է, որն աղտահարում է սնունդն ու կերերը, և դասակարգվում որպես մարդու համար հնարավոր կանցերոգեն: Մեր կատարած աշխատանքում ներկայացված է OTA (4.375 մկգ/կգ/օրը) գենաթունային ակտիվությունը Վիստար առնետների ոսկրածուծի բջիջներում, որոնք ստացել են միկոտոքսինը կերի միջոցով 15, 30 և 60 օր: ԴՆԹ-ի վնասվածքների մակարդակը գնահատվել է առանձին բջիջների ժել-էլեկտրաֆորեզի (ԴՆԹ-կոմետ) –ստանդարտ հիմնային մեթոդի կիրառմամբ: Պոչի ինտենսիվությունն ու պոչի մոմենտը հավաստիորեն ($p<0.05$) բարձր էին 15 և 60 օր մշակված կենդանիների խմբերում համեմատած ստուգիչի հետ: ԴՆԹ-ի վնասվածության այս չափորոշիչները հավաստիորեն ($p<0.05$) բարձր էին նաև 30 օր մշակված կենդանիների խմբի հետ համեմատած: Պոչի ինտենսիվության և պոչի մոմենտի առավել բարձր մակարդակները դիտվել են 15 օր միկոտոքսինով մշակված կենդանիների խմբում, որոնք հավաստիորեն ($p<0.05$) տարբերվում էին 60 օր մշակվածներից: Մեր ստացած տվյալները հաստատում են OTA գենաթունային էֆեկտները և ցույց տալիս դրա ակտիվությունը ցածր չափաբաժիններում առնետների ոսկրածուծի բջիջներում ԴՆԹ-կոմետ մեթոդի կիրառմամբ:

Օխրատոքսին A – միկոտոքսիններ – գենաթունային ակտիվություն – առնետի ոսկրածուծի բջիջներ – ԴՆԹ-կոմետ մեթոդ

Охратоксин А (OTA) является микотоксином, являясь распространенным контаминантом продуктов питания и кормов, классифицируется как потенциальный канцероген для человека. В представленной работе изучена генотоксическая активность OTA (4.375 мкг/кг/день) в клетках костного мозга крыс линии Вистар, получавших микотоксин вместе с пищей в течение 15, 30 и 60 дней. Уровень повреждений ДНК оценивали методом гельэлектрофореза в единичных клетках (ДНК-комет). Достоверное ($p<0.05$) повышение уровня интенсивности и момента хвоста наблюдали у крыс, обработанных в течение 15 и 60 дней, по сравнению с контролем. Данные параметры были также достоверно ($p<0.05$) выше по сравнению с 30-дневной обработкой. Интенсивность и момент хвоста были наиболее вы-

ражены в группе крыс, обработанной в течение 15 дней, и были достоверно ($p<0.05$) выше по сравнению с 60-дневной обработкой. Полученные результаты подтверждают генотоксический потенциал ОТА и показывают его активность в низких концентрациях в клетках костного мозга крыс методом ДНК-комет.

Охратоксин А – микотоксины – генотоксическая активность – клетки костного мозга крыс – метод ДНК-комет

Ochratoxins are fungal secondary metabolites produced by several species of *Aspergillus* and *Penicillium*. Most studies on ochratoxins have focused on ochratoxin A (OTA) [1]. Though there are few studies of OTA genotoxicity in experimental animals and the results obtained with cell cultures are inconsistent [2], nowadays it is known that this mycotoxin possesses mutagenic, teratogenic and nephrotoxic activity [3]. The genotoxic status of OTA is still controversial because contradictory results were obtained in various microbial and mammalian gene mutation assays [4].

Most people have detectable levels of OTA in the bloodstream (at least in certain countries), though usually at very low levels. OTA was detected in 100% of human blood samples (maximum 0.04 μg/L) and 58 % of human milk samples (maximum 0.9 μg/L) in Norway [5]. The International Agency for Research on Cancer classified OTA as a 2B group compound (possibly carcinogenic to humans and with sufficient evidence for carcinogenicity in laboratory animals), though the mechanism of its carcinogenicity is not understood completely [6].

The available reports on genotoxicity tested by the DNA-comet assay (single-cell gel electrophoresis) in rat demonstrate the increase of the level of DNA damage in kidney and liver cells, but animals were treated only with a high oral OTA doses (0.5 mg/kg body weight, 10 mg/kg body weight) [2, 7, 8]. Comet assay is a sensitive and operative method for determining DNA strand breaks and alkali labile sites at the cell level *in vitro* and *in vivo* [9]. The method does not require cell cultivation, which makes easier analysis of genotoxicity of different agents in the target tissue.

To our knowledge there is no data on genotoxicity of OTA chronic exposure in rat bone marrow cells in low dose, so the aim of our research is to analyze these effects *in vivo*.

Materials and methods. Adult Wistar rats weighing 200±20g were kept in standard environmental conditions with a 12-h light/dark cycle and at a constant temperature of 24°C, fed a standard diet and had free access to water. The study was approved by the Ethical Committee of the Institute of Molecular Biology of the NAS RA (IRB IORG0003427).

The rats were randomly assigned to four groups of 5 animals each receiving mycotoxin OTA (4.375 μg/kg/day) or solvent only (control group) for 15, 30 and 60 days. The doses of mycotoxins were selected on the base of literature data related to genotoxicity of OTA in rats [7, 8]. 5 rats for each variant were anesthetised by chloroform. For evaluation of genotoxic activity of OTA the left femur was removed and the bone marrow at both ends was exposed with bone cutters. Cells were flushed out with 3 mL PBS (pH 7.4) using a needle and syringe, and the cell suspension was filtered through a three layer bolting cloth [10].

Comet assay. As an anticoagulant heparin was used in ratio 1ml/0.3ml (blood/anticoagulant). The level of DNA damage was evaluated by standard comet assay method [8]. 20μl of cell suspensions mixed with 0.5% low-melting agarose (80μl) were added to slides pre-coated with 1% normal-melting agarose. After the solidification of gel layer the slides were immersed in a lysis solution (2.5 mol/l NaCl, 100 mmol/l EDTA disodium salt (pH 8.0), 10 mmol/l Tris buffer (pH 10.0) and 1% Triton X-100) at $+4^0$C for 60 min. Slides were placed in electrophoresis buffer (0.3 M NaOH, 1 mM Na_2EDTA, pH 13) for 40 min to allow DNA to unwind. Electrophoresis was performed for 20 min at 300 mA and 1 V/cm. Slides were neutralized with Tris-HCl buffer, pH 7.5, and stained with 20 μg/mL ethidium-bromide.

Slides were examined at 250× magnification on a fluorescent microscope (ZEISS, Germany). At least 150 cells were scored per animal (50 cells scored per each of three replicate slides). Images of comets were recorded with a video camera with high sensitivity (Variocam, PCO, Germany) and processed on a computer program Comet Assay IV (Version 4.3). Tail moment and tail intensity are used to evaluate the extent of DNA damage.

Statistical analysis of the results was performed using SPSS 19 software package with application of non-parametric Mann-Whitney test (U test).

Results and Discussion. We assessed levels of DNA damage and the time course of OTA genotoxicity in the bone marrow cells of animals. The main parameters of DNA damage: tail intensity and tail moment have increased significantly ($p<0.05$) after 15 and 60 days treatment in comparison to control. These effects was not observed in the group treated for 30 days (fig. 1).

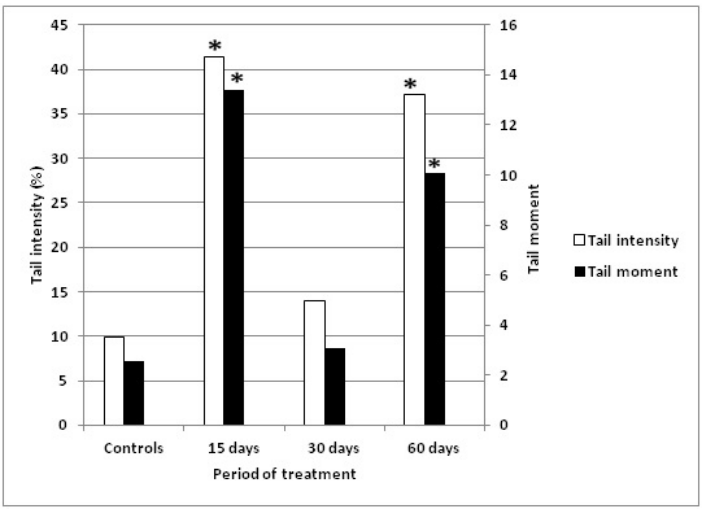

Fig. 1. Levels of DNA damage in rat bone marrow cells after chronic exposure evaluated by comet assay. *$p<0.05$ - significant difference in comparison to control.

The highest levels of DNA damage were observed after 15 days of treatment. Levels of tail intensity and tail moment are decreasing after 30 days and increasing significantly ($p<0.05$) after 60 days treatment. These effects could be due to activation of protective mechanisms of organism (e.g. binding and excretion of OTA and its metabolites), which later (after 60 days treatment) failed to prevent genotoxicity of OTA after long term exposure to low dose of mycotoxin. In addition, we showed that comet assay is sensitive method to evaluate genotoxic effects of OTA in rat bone marrow cells after chronic exposure.

Acknowledgements

The work was supported by State Committee of Science of RA (grant#11-1f 329). I also would like to express my gratitude to Dr. A. Karapetyan (YSU, Department of Zoology) for support during conduction of experiments on rats, as well as to MetaSystems GMBH company for their permanent technical support.

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Received on 09.10.2014