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Protective role of chlorogenic acid on DNA damage caused by ochratoxin A exposure

Rol protector del ácido clorogénico sobre el daño del ADN causado por la exposición a ocratoxina A

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Abstract

Chlorogenic acid (ChlA) has shown short-term protective effects against the cytogenotoxic effects of ochratoxin A (OTA). The present study evaluated the effect of oral administration of ChlA in male Wistar rats exposed to OTA. OTA (0.4 mg/kg bw/day), ChlA (5 mg/kg bw/day), or the combination of both, were administered orally to animals during 28 days. No deaths, decrease in feed consumption or change in the body weight of animals were observed in any group. In the OTA-treated group a decrease in locomotion as well as increased DNA damage in blood, kidney and bone marrow cells were observed. ChlA alone was not genotoxic for animals. The combination of OTA +ChlA decreased the DNA damage by 37% in blood cells, by 55% in kidney cells and by 80% in bone marrow cells compared to OTA-treated group. In conclusion, oral treatment with ChlA showed a good protective effect on genotoxicity produced by OTA in rats during 28 days exposure.

Keywords: ochratoxin A, genotoxicity, chlorogenic acid.

Resumen

El ácido clorogénico (ChlA) mostró efectos protectores a corto plazo contra los efectos citogenotóxicos de ocratoxina A (OTA). En el presente estudio se evaluó si este polifenol mantiene su efecto protector mediante la administración oral en ratas Wistar machos expuestas a OTA. OTA (0,4 mg/kg/día), ChlA (5 mg/kg/día), o la combinación de ambos, fueron administrados a los animales por vía oral durante 28 días. No se observaron muertes, ni disminución en el consumo de alimento, ni cambios en el peso corporal en ningún grupo de animales. En el grupo tratado con OTA se observó disminución en la locomoción, así como daño en el ADN en las células de sangre, riñón y médula ósea. En los animales que solo recibieron ChlA no se observaron efectos genotóxicos. La combinación de OTA + ChlA disminuyó el daño del ADN en un 37% en las células sanguíneas, en un 55% en las células renales y en un 80% en las células de la médula ósea con respecto al grupo tratado con OTA. En conclusión, el tratamiento oral con ChlA mostró buen efecto protector sobre la genotoxicidad producida por OTA en ratas durante 28 días de exposición.

Palabras clave: ocratoxina A, genotoxicidad, ácido clorogénico.

Introduction

Ochratoxin A (OTA) is a toxic secondary metabolite produced by species of filamentous fungi of the genera *Penicillium* and *Aspergillus*, which grow on a wide range of organic substrates. This mycotoxin is present mainly in cereals for human consumption and animal feed as well as in alcoholic beverages, coffee and cocoa (Ravelo Abreu *et al.*, 2011). Generally, monogastric and younger animals are more sensitive to mycotoxins than ruminant or older animals. The high ingestion of mycotoxins can cause deterioration of animal health as well as decreased production. Mycotoxicosis occurs with decrease in feed consumption and increase in morbidity and mortality rates (Muzaffer & Pérez, 2006). The nephrotoxic, hepatotoxic, embryotoxic, teratogenic, neurotoxic, immunotoxic, genotoxic, and carcinogenic effects of OTA have already been demonstrated (Wang *et al.*, 2020).

Polyphenols constitute one of the largest groups of non-energetic substances present in plants and foods of plant origin, with antioxidant properties that account for many of their beneficial effects (Quiñones *et al.*, 2012). Different studies report that the toxic effects of mycotoxins could be reduced by polyphenols (Mazur-Kuśnirek *et al.*, 2019). Chlorogenic acid (ChlA) is an important antioxidant polyphenol which is found in certain plants and is the main phenolic compound in coffee (Tajik *et al.*, 2017). The protective effect of ChlA on cyto-genotoxicity induced by different toxic substances has been reported (Carranza-Torres *et al.*, 2019). A previous study reported the short-term protective effects of ChlA on DNA damage induced by OTA in Balb/c mice after 24 hours of intraperitoneal treatment (Cariddi *et al.*, 2015). The present study aimed to determine the possible protective effect of orally administered ChlA on DNA damage in rats exposed to OTA for 28 days by means of micronucleus assays in bone marrow cells and comet assays in blood and kidney cells.

Materials and methods

Reagents

Ochratoxin A was purchased from BioPure Technology (UK); dimethylsulfoxide (DMSO) was obtained from Merck (Germany) and chlorogenic acid ($\geq 95\%$) was purchased from Sigma St. Louis, (USA). The doses of OTA (0.4 mg/kg bw/day) and ChlA (5 mg/kg bw/day) used, were based on a previous study (Cariddi *et al.*, 2016). OTA and ChlA were dissolved in DMSO Merck (Germany)/phosphate buffered solution (PBS) (solvent concentration not exceeding 0.03%).

Experimental animals and treatments

All experimental procedures were conducted in accordance with recent legislation. This study was approved by the *Comité de Ética de la Investigación Científica* (COEDI), Universidad Nacional de Río Cuarto (File No. 73/12) Male Wistar rats were used. The trials were performed with male rats because of their high susceptibility to OTA renal damage (Vettorazzi *et al.*, 2014). Ten to twelve week old male Wistar rats (weighing 150-200 g), supplied from *Bioterio of the Universidad Nacional de Río Cuarto*, were maintained in a temperature and humidity controlled room, with 12 hours light-dark cycles and food and water *ad libitum*. Briefly, animals were randomly divided into four groups (four animals per group; n=4) and treated individually by daily intragastric gavage during 28 days as follows: group 1: 0.2 ml/day DMSO/PBS (Vehicle Control Group), group 2: OTA (0.4 mg/kg bw/day; OTA), group 3: ChlA (5 mg/kg bw/day; ChlA), and group 4: a combination of OTA (0.4 mg/kg bw/day) and ChlA (5 mg/kg bw/day; OTA+ChlA). On day 29, animals were euthanized and blood samples were collected immediately with heparin for comet assay. The kidneys were dissected and stored at -20 °C until further analysis by comet assay and the femurs were excised for micronucleus assay.

Toxicity parameters

The animals were examined once daily in order to observe toxicity reactions (hunched posture, waggling gait, decreased locomotion, aggressive behavior) and feed consumption. Body weight was recorded once a week.

Comet assay for blood samples

The heparinized blood samples were kept in the dark at 4 °C and processed within 6 hours. The comet assay was performed as described by Cariddi *et al.* (2015).

Comet assay for kidney samples

Frozen kidney tissues were thawed at room temperature during preparation, and placed in cylindrical sieves which were filled with 1.5 ml ice-cold Merchant's medium (0.14 M NaCl, 1.47 mM KH₂PO₄, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 10 mM Na₂EDTA, pH 7.4) The comet assay was performed according to the protocol proposed by Jackson *et al.* (2013). Tissues were homogenized by pressing through sieves as the plunger was moved up and down several times to obtain a cell suspension. The cells were trypsinized and suspended in PBS at a density of approximately 1×10⁵ cells/ml. A 50 µl aliquot of each cell suspension was added to 100 µl of 0.75% low melting point agarose at 37 °C. The mixtures were layered onto slides pre-coated with 0.75% normal melting point agarose and covered with a coverslip. From this point forward, the protocol was identical to that used for the comet assay on blood samples.

Micronucleus assay

The assay was performed in bone marrow cells following standard protocols described by Cariddi *et al.* (2015).

Statistical analysis

Results were evaluated using GraphPad Prism version 5.00.288 (San Diego, USA, 2007) and compared with one-way analysis of variance (ANOVA) and the Tukey multiple comparison test or Kruskal Wallis test and Dunns multiple comparisons. The differences were considered to be statistically significant at p<0.05.

Results

No deaths were recorded in any experimental group during the 28 days of the *in vivo* assay. However, the OTA-treated group showed decreased locomotion and overall less movement than other treatment groups. Rats treated with ChlA, OTA+ChlA and DMSO/PBS did not show reactions of toxicity. Neither decrease in feed consumption nor changes in body weight gain of animals were observed in any group (Figures 1 A and B).

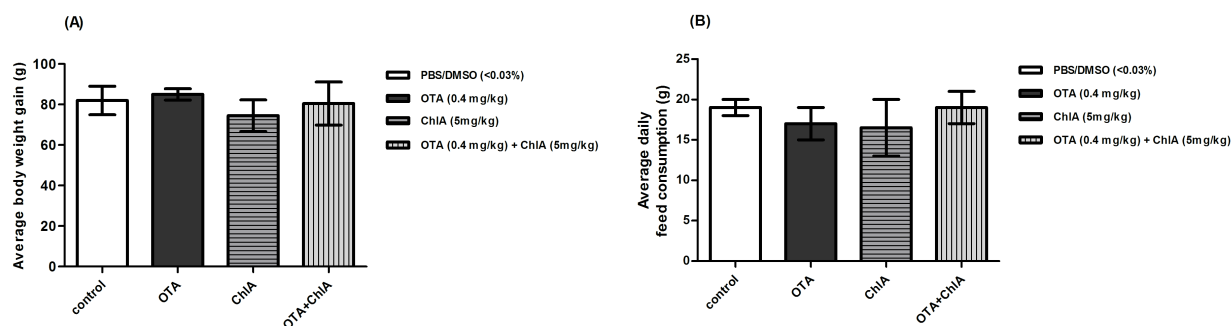


Figure 1. A) Average daily body weight gain and B) Average daily feed consumption of Wistar rats in the 28 days of treatment with ochratoxin A (OTA), chlorogenic acid (ChIA) or combination of OTA+ ChIA. Four rats were used per experimental group. Each value represents means $\bar{A} \pm$ SD. DMSO: dimethylsulfoxide. Statistical test: ANOVA and Tukey's Test.

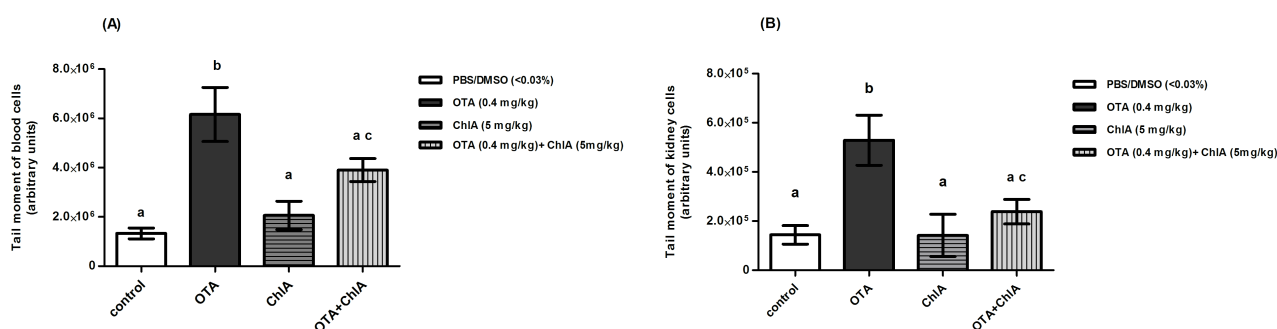


Figure 2. Protective effects of chlorogenic acid (ChIA) on ochratoxin A (OTA)-induced DNA damage (tail moment) measured by comet assay. A) blood cells or B) kidney cells from Wistar rats (n = 4) after 28 days of treatment. Each value represents the means \pm SD. Statistical test: Kruskal-Wallis and Dunns Test. c: $p < 0.05$ compared with OTA group.

Figures 2 A and B show the results of the comet assay. In OTA-treated group an increase in tail moment of the blood and kidney cells compared to control group ($p < 0.001$) was observed. However, treatment of the animals with OTA+ChIA produced a decrease in DNA damage by 37% in blood cells and by 55% in kidney cells ($p < 0.05$) with respect to OTA. The micronucleus assay results are shown in Table 1. An increase in the frequency of micronucleus (MN) in polychromatic erythrocytes (PCE) in OTA-treated group compared to control group ($p < 0.001$) was observed. The frequency of MN in PCE was reduced by 80% with OTA+ChIA acid treatment compared to OTA-treated group ($p < 0.001$). No differences were observed in the PCE/normochromatic erythrocytes (NCE) ratio in any group. Cellular DNA of animals treated with only ChIA was not altered.

Table 1. Protective effects of chlorogenic acid (ChIA) on ochratoxin A (OTA) induced genotoxic effects on bone marrow erythrocytes from Wistar rats after 28 days of treatment.

Treatments	Wistar rats (n)	TI (PCE/NCE± SD)	GI MNPCE (%)
Negative control (DMSO/PBS)	4	1.38 ± 0.50	8.50 ± 2.12 ^a
OTA (0.4 mg/kg bw)	4	1.43 ± 0.16	63.00 ± 12.73 ^b
ChIA (5 mg/kg bw)	4	1.33 ± 0.24	12.50 ± 2.12 ^a
OTA (0.4 mg/kg) + ChIA (5 mg/kg)	4	1.41 ± 0.29	12.50 ± 0.71 ^a

Four rats were used per experimental group; TI: Toxicity Index; GI: Genotoxicity Index; PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; MNPCE: micronucleated polychromatic erythrocytes. Statistical test: ANOVA and Tukey's Test. Different superscript letter indicates statistical significance.

Discussion

This work evaluated the protective effect of ChIA administered orally on DNA damage in Wistar rats exposed to OTA. Body weight is an important indicator of toxicity; however, this parameter was not altered in any treatment group. In a previous study we observed similar results (Cariddi *et al.*, 2016). In the present study we reported the DNA damage induced by OTA on blood cells and bone marrow cells, as reported previously by our laboratory (Cariddi *et al.*, 2015) as well as by other authors (Corcuera *et al.*, 2015). We also found DNA damage in kidney cells of animals treated with OTA, as demonstrated by other researchers (Abdel-Wahhab *et al.* 2017). Some studies have related OTA damage in kidney with carcinogenicity (Tajik *et al.*, 2017). The protective effect of ChIA on DNA damage induced by OTA was already demonstrated in a previous study (Cariddi *et al.*, 2015). However, in that study both polyphenol and mycotoxin were administered intraperitoneally, and the protective effect was evaluated 24 hours post-injection. In the present study, oral administration of ChIA during 28 days in rats exposed to OTA also protected from the genotoxicity induced by this mycotoxin. Chlorogenic acid is a powerful natural antioxidant (Xu *et al.*, 2012). In this study, oral treatment of rats with only ChIA did not alter DNA of cells. Other studies have also demonstrated the lack of toxicity of this compound (Wang *et al.*, 2020). In a previous study in rats exposed to OTA we demonstrated that the oral administration of ChIA reversed the pathological damage induced by this mycotoxin in kidneys, as well as the biochemical parameters altered in urine and serum. However, we did not observe an increase in the oxidative stress parameters measured in kidneys of OTA-treated animals, indicating a protective effect of ChIA but not by an antioxidant mechanism (Cariddi *et al.*, 2016). Although some studies report oxidative DNA damage induced by OTA in kidneys

(Abdel-Wahhab *et al.*, 2017), this is controversial because several authors demonstrated a lack of oxidative damage associated to the toxic effects caused by this mycotoxin (Kószegi & Poór, 2016). The results suggest that ChIA could be protecting cellular DNA from the damage induced by OTA by a different mechanism.

In conclusion, oral treatment with ChIA decreased DNA damage induced by OTA exposure in blood, bone marrow and kidney cells showing a good protective activity that may be important for the prevention of ochratoxicosis in susceptible animals.

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Competing interests

The authors declare that there are no conflicts of interest.

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