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Effects of resveratrol on 8-OHdG levels and endogenous antioxidants evaluated simultaneously with micronucleus and apoptosis frequencies in peripheral blood of mice treated with hexavalent chromium

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Abstract

Cr(VI) compounds generate reactive oxygen species during reduction to Cr(III) leading to DNA damage such as 8-hydroxydeoxyguanine (8-OHdG). Resveratrol (RV) has received much attention for its ability to reduce stress-induced cellular injury. The effects of RV on superoxide dismutase (SOD) activity, 8-OHdG and glutathione (GSH) levels, as well as on micronucleus (MN) and apoptosis frequencies, were evaluated simultaneously. Groups of five Hsd-ICR mice were treated as follows: i) vehicle only; ii) RV (50 mg/kg) by gavage; iii) CrO_3 (20 mg/kg) intraperitoneally; and iv) RV in addition to CrO_3. Evaluations were performed on samples of peripheral blood obtained at 0h, 24h, 48h and 72h after treatments. No significant changes in MN or apoptosis were observed in the group treated with RV only, although the 8-OHdG, GSH and SOD were modified. Treatment with CrO_3 increased MN and apoptosis and decreased SOD and 8-OHd. The treatment with RV prior to administration of CrO_3 increased apoptotic cell frequencies and 8-OHdG levels, suggesting that these may play a role in the elimination and reduction of micronucleated cells. The decreased GSH, and the recovery of SOD by the RV could be related with its oxidative stress-suppression properties on genotoxic damage induced by CrO_3.

Introduction

Polyphenols such as resveratrol (RV) are known for their cytoprotective actions against several diseases related to oxidative stress. RV is a polyphenolic component contained in grapes and red wine. The biological effects of RV may be explained in part by its antioxidant properties due to the presence of the phenolic hydroxyl groups in its chemical structure (Leonard et al., 2003). The antioxidant properties of RV could be mediated by a) its ability to scavenge free radicals, and b) its ability to promote the activities of

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antioxidants such as glutathione (GSH) and antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Li et al., 2006). Thus, substances with antioxidant properties have emerged as putative preventatives and co-adjuvants in the treatment of chronic degenerative diseases related to oxidative stress and DNA damage (García-Rodríguez et al. 2013). According to previous studies, Cr(VI)-induced genomic DNA damage includes 8-hydroxydeoxyguanosine (8-OH-dG) (Maeng et al., 2003), which is a form of oxidative DNA damage. Therefore, in this study we evaluated the effects of RV on SOD activity, 8-OHdG and GSH levels, as well as micronucleus (MN) and apoptosis frequencies simultaneously in order to identify and understand the possible beneficial effects of RV against Cr(VI)-induced genotoxicity.

Materials and Methods

Chemicals and reagents

Chromium trioxide (CrO_3; CAS 1333-82-0), acridine orange (AO; CAS 10127-02-3), ethidium bromide (EB; CAS 1239-45-8), and 3,4′,5-trihydroxystilbene (RV; CAS 501-36-0) were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals

Two- to 3-month-old male Hsd:ICR mice weighing 28-35 g were used in the experiments. The animals were maintained in a temperature-controlled environment (22°C) with a 12/12-h light–dark cycle (light 07:00 19:00 h). Mice had free access to food (Purina-Mexico® chow for small rodents) and water. All mice were obtained from Harlan® Mexico, and acclimated for a 2-week period. The Bioethics Committee of the "Facultad de Estudios Superiores-Zaragoza, UNAM", approved the experimental conditions and protocols.

Experimental design

The CrO_3 and RV doses were selected according to previous investigations in which 20 mg/kg of CrO_3 administered ip elevated the number of MN (García-Rodríguez et al., 2001), and 50mg/kg of RV did not induced genotoxic damage (Bisht et al. 2010). The solutions of CrO_3 and RV were prepared by dissolving dry compounds in sterile distilled water and ethanol-30% respectively. Solutions (0.25 ml) were administered immediately following their preparation. The control group was treated in an identical manner (ip or gavage) with the vehicle only. The evaluation criteria and working conditions were conducted according to guidelines of the Collaborative Study Group for the Micronucleus Test (CSGMT) (Heddle et al., 1983). After establishing treatment doses, the effects of RV on genotoxic damage induced by CrO_3 were evaluated. This assessment was performed using MN kinetic analysis. Mice were assigned at random to one of the following groups (n = 5 mice per group): (1) animals treated with vehicle only (C1; vehicle water, distilled), (2) animals treated with vehicle only (C2; vehicle ethanol-30%), (2) animals treated with RV (50mg/kg) by gavage, (3) animals injected with CrO_3 (20mg/kg), and (4) animals treated with RV (50mg/kg) by gavage and 4 h later injected with CrO_3 (20mg/kg).

Micronucleus (MN) assay

Slides were prepared by coating them with AO according to the technique described by Hayashi et al. (1994). To evaluate the frequencies of MN after the treatments, 5-µl peripheral blood samples were collected by piercing a tail blood vessel of each mouse every 24h during a 4-day period (0 to 72 h). Samples were placed directly on slides previously treated with AO. The MN analysis was based on 4000 polychromatic erythrocytes per mouse, where the presence of MN was considered genotoxic damage. The evaluations were conducted by identifying normochromatic erythrocytes (NCE), polychromatic erythrocytes (PCE), and MN in PCE with a fluorescent microscope (Nikon OPTIPHOT-2) using blue excitation (480 nm) and a barrier filter (515–530nm) at 100x magnification. In parallel, the relative proportions of the PCE and NCE (1000 cells) were determined.

Apoptosis and cell viability analyses

To evaluate apoptosis and cell viability, differential AO/EB staining was used according to the technique previously adapted for peripheral blood (García-Rodríguez et al., 2013). Blood samples (100 µl) were collected by piercing a blood vessel in the tails of mice prior to treatment and 48h post treatment.

Two slides were prepared per mouse, and analysis was conducted immediately. The apoptotic and cell viability assessments were based on 300 cells per

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mouse. Apoptotic and necrotic cells were identified using a fluorescent microscope (Nikon OPTIPHOT-2) using blue excitation (480 nm) and a barrier filter (515–530 nm) at 40x magnification.

Apoptosis, necrosis, and cell viability were evaluated directly in the peripheral blood of mice 48h post treatment. The CrO_3 group was considered a positive control according to previous studies, where it was found that CrO_3 induces apoptosis (García-Rodríguez et al., 2013). The cytotoxic effects were determined by PCE/NCE ratio, apoptosis, necrosis, and cell viability directly in peripheral blood cells.

Statistical analysis

The MN-PCE induction results are presented as Net Induction Frequency (NIF) of MN, while apoptotic and necrotic cells are expressed as percentages. These evaluations were analysed with chi-squared tests. SOD activity, 8-OHdG and GSH levels are expressed as means ± standard deviation (SD). Results from various treatment groups were subjected to analysis of variance (ANOVA) followed by Tukey's test (Heddle et al., 1983; García-Rodríguez et al., 2001). SPSS/PC V18TM and Statistica/PC V 6.0TM software were used for statistical analyses. For all of the analyses, $p<0.05$ was considered to be significant.

Results and Discussion

The averages of MN-PCE frequencies are represented as "the absolute value" and were analyzed as follows:

NIF = |average of MN-PCE frequencies measured at time x_i
− average of MN-PCE frequencies measured at time 0|

Where x_i is the evaluation at 24h, 48h, or 72h per group and time 0 is evaluation at 0 h (before treatment) per group.

Calculation of the NIF improves the ability to determine net MN-PCE induction. This calculation subtracts the frequency of MN-PCE prior to treatment from the frequency following treatment, thereby eliminating baseline MN-PCE variability that occurs among treatment groups at time 0.

Figure 1 presents the NIF of MN-PCE values for all treatments at 24h, 48h, and 72h post treatment. The RV did not affect the frequency of MN-PCE in treated mice. The genotoxicity of Cr(VI) was demonstrated by a significant increase in MN-PCE occurrence at 24h, 48h and 72h post treatment in the CrO_3-treated group. This rise, noted in all samples, corroborates previous findings (García-Rodríguez et al., 2001). The mechanism underlying the genotoxicity of Cr(VI) compounds could be linked to intracellular reduction of Cr(VI) to Cr(III). During this chemical process, reactive oxygen species (ROS) and free radicals are generated (Valko et al., 2006), which may lead to formation of MN-PCE. Although the direct relationship between DNA-ROS and Cr(VI)-induced DNA damage is not completely resolved, it is possible that ROS play a role in Cr(VI)-induced MN.

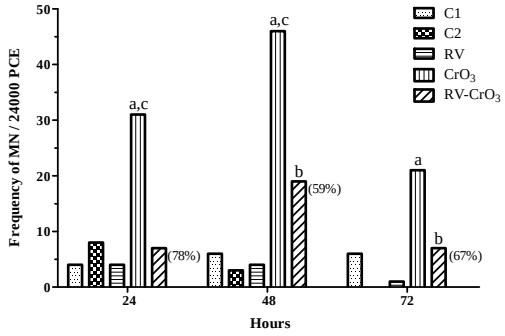

Figure 1. *Effect of RV on the MN-PCEs (% reduction). Data show MN-PCE frequencies obtained at 24h, 48h and 72h minus the MN-PCE frequency at 0h after treatment with CrO_3. C1, vehicle water, distilled; C2, vehicle ethanol-30%. $p<0.05$. [a]vs. C1; [b]vs. C2; [c]vs. RV-CrO_3.*

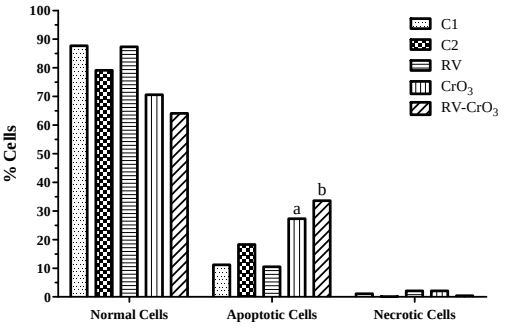

Figure 2. *Frequency of early, late apoptotic and necrotic cells in peripheral blood, evaluated at 48h after treatment with CrO_3. C1:vehicle water, distilled; C2: vehicle ethanol-30%. $p<0.05$. [a]vs. C1; [b]vs. C2.*

When treatment included both RV and CrO_3, a decrease in number of MN-PCE was observed at 24h, 48h, and 72h post treatment (78, 59 y 67% respectively) compared to CrO_3 alone. In our evaluation of apoptosis, both the CrO_3 and RV-CrO_3

treatments showed a significant increase in the frequency of apoptotic cells compared to the control, and was highest in the group that received both treatments (RV-CrO_3) (*Figure 2*). The increase of apoptotic cells in this group suggests that these may play a role in the elimination and reduction of micronucleated cells.

In the evaluations of 8-OHdG levels and endogenous antioxidants, the group treated with RV showed increased SOD activity. However, in the groups treated with CrO_3 and RV-CrO_3, SOD activity decreased, more significantly in the CrO_3 group. In addition, treatment with RV prior to administration of CrO_3 decreased GSH and increased 8-OHdG levels (*Figure 3*). The increase of 8-OHdG levels in this group suggests that RV may play a role in the elimination of DNA damage (García-Rodríguez et al., 2017), and the decreased GSH levels, and the recovery of SOD activity by the RV could be related with its oxidative stress-suppression properties on genotoxic damage induced by CrO_3.

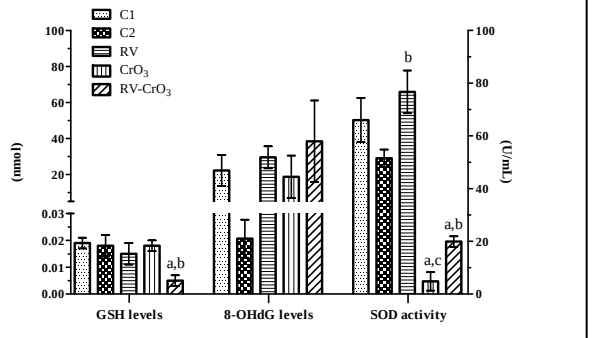

Figure 3. *Average GSH and 8-OHdG levels and SOD activity in peripheral blood 48h after treatment. C1:vehicle water, distilled; C2:vehicle ethanol-30%. p<0.05; [a]vs. C1; [b]vs. C2; [c]vs. RV-CrO_3.*

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