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Effect of resveratrol on chromosomal aberrations induced by doxorubicin in rat bone marrow cells

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ABSTRACT

This study investigated the effects of resveratrol (RES) on doxorubicin (DXR) induced rat bone marrow cell chromosome aberrations. RES, a polyphenolic compound, has attracted considerable attention because of its antioxidant and antimutagenic effects. DXR, a chemotherapeutic agent, is known to cause chromosomal aberrations in healthy cells in cancer patients. In this study, Wistar albino male rats were divided into 6 groups with 6 animals each. The control group received distilled water i.p. and the DXR group received an i.p. injection of doxorubicin (90 mg/kg bw). For the 2 RES dose groups (12.5 and 25 mg/kg bw, respectively), RES was injected i.p. 5 times during the 24 h study period to coincide with the schedule for the DXR + RES groups. The DXR-RES groups received DXR (90 mg/kg bw) and RES at either 12.5 or 25 mg/kg bw, i.p. 30 min before, concurrently, and then every 6 h after DXR administration. Bone marrow collection was timed to coincide with 24 h after DXR administration in all groups. RES administration alone did not induce any significant increase in frequency of chromosome aberrations or abnormal metaphases compared with controls ($p > 0.05$) while DXR alone did ($p < 0.05$). In the DXR-RES 12.5 mg/kg bw group, frequency of chromosome aberrations and abnormal metaphases were slightly reduced compared to DXR alone, but this was not statistically significant. However, in the DXR-RES 25 mg/kg bw group, RES resulted in a statistically significant reduction in the frequency of chromosome aberrations and abnormal metaphases compared to those induced by DXR alone ($p < 0.05$). These results indicate that RES (25 mg/kg bw) significantly reduces frequency of DXR induced chromosome damage in bone marrow cells.

1. Introduction

Doxorubicin (DXR) is a chemotherapeutic agent used to treat breast cancer, Hodgkin's and non-Hodgkin's lymphomas, osteosarcomas, and leukemias [1,2]. The cytotoxic effect of DXR is mediated by binding to DNA, inhibiting enzymes required for DNA replication, such as Topoisomerase II (TopoII), or indirectly through generation of free radicals [3,4]. These cytotoxic effects provide anti-cancer activity but also lead to serious side effects in organs such as heart, brain, and kidney [5].

DXR binding to tyrosine residues of Topo II inhibits DNA replication and results in DNA double-strand breaks [6]. DXR also binds to DNA via intercalation [7], resulting in chromosome aberrations. Reduction of DXR by a membrane-bound reductase enzyme leads to production of reactive species, such as DXR quinone and semiquinone, superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), and peroxynitrite ($ONOO^-$) [8]. These species can oxidize protein, lipids, and nucleic acids and potentially cause DNA strand breaks. Lipid peroxidation results in malondialdehyde (MDA) production. MDA interactions with DNA can generate alkylated DNA adducts that inhibit DNA replication [8] and again, lead to chromosomal damage. As a result, direct and indirect actions of DXR can induce mutations and chromosome aberrations in normal and tumor cells. These clastogenic effects involve chromosome and chromatid damage, sister chromatid exchanges, complex figures (triradial, quadriradial, dicentric), decreased mitotic index, and increased frequency of abnormal metaphase cells [9,10].

Resveratrol (RES; *trans*-3,5,4′-trihydroxystilbene) is a polyphenolic compound found abundantly in grape skins and other plants,

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including berries, peanuts, and *Polygnum cuspidatum* [11]. RES has anti-cancer, anti-genotoxicity, anti-inflammatory, anti-apoptotic, and anti-oxidant effects that are mediated through molecular mechanisms dependent on dose, contact time, and cell type [12]. RES has inhibitory effects in many types of cancer, e.g. prostate, lung, breast, colon, skin, and pancreatic cancers, leukemia, and lymphoma [13]. It may exert its anti-cancer action through activation of p53 and Bax, downregulation of Bcl2 and Bcl-XL [14–16], or inhibition of transcription of NFκB, enhancing apoptosis via p53 activation [17]. RES can effectively sensitize tumor cells to antineoplastic drugs [18]. RES enhances the chemotherapeutic potential of DXR in B16 melanoma, myeloid leukemia, pancreatic cancer, and gynecologic cancer cells [19–22]. RES also has a protective effect against apoptosis and necrosis induced by reactive oxygen species or hydrogen peroxide in rat hepotocytes and pheochromocytoma [23,24].

The antioxidant effects of RES include radical scavenging activity, reducing ability, and Fe^{2+} chelating activity [25]. RES exhibits a protection effect against hydroxyl, superoxide and metal-induced radicals in cellular systems [26]. RES can exert antioxidant effects by reducing DNA oxidation and DNA fragmentation induced by oxidative stress [27–29]. RES can significantly decrease lipid peroxidation in rodent erythrocytes, plasma, and brain [30,31]. However, this effect may be tissue specific. One study reported that RES had protective effects on heart lipid peroxidation caused by DXR, but did not show any protection in rat liver [32]. RES also regulates detoxication, by increasing the activation of enzymes such as catalase, superoxide dismutase (SOD), glutathione reductase (GR), NADPH quinone oxidoreductase, and glutathione S-transferase (GST) [33].

RES can cause cell cycle arrest at S phase through the promotion of cdk1 phosphorylation [34], and blocking DNA synthesis by inhibiting DNA polymerase and ribonucleotide reductase [35]. RES can also cause cell cycle arrest at G2/M through inhibition of cdk7, which plays a role in cdk1 activation [36].

Can administration of RES modify DXR-induced clastogenic effects on normal cells? The present study was undertaken to investigate the modulatory effects of RES and its combination with DXR on the clastogenic action of DXR in Wistar rat bone marrow cells.

2. Materials and methods

2.1. Chemicals

Doxorubicin (DXR; Adriablastina, CAS 23214-92-8, Kocaeli, Turkey) was dissolved in distilled water (10 mg/ml) and administered by single intraperitoneal injection at a dose of 90 mg/kg body weight (bw). Resveratrol (CAS 501-36-0, Sigma–Aldrich, Istanbul, Turkey) was dissolved in 95% ethanol to a concentration of 50 mg/ml. Prior to injection, the RES/ethanol stock was diluted with distilled water (1:9) for a final concentration of 5 mg/ml and administered by intraperitoneal injection at doses of 12.5 and 25 mg/kg bw. All other chemicals and reagents used were of analytical (Sigma–Aldrich) grade.

2.2. Animals and experimental design

Six-week-old male Wistar rats (*Rattus norvegicus*) with an average body weight of 150 g were used. Animals and their standard diet were supplied by the Experimental Medical Research Unit, Faculty of Medicine, Kocaeli University (Kocaeli, Turkey). Animals were housed in a standard animal care facility with room temperature 23 ± 2 °C and 12 h light/dark cycle. They had free access to standard rat chow and fresh water ad libitum. The Ethics Committee of Kocaeli University School of Medicine approved the protocol.

The dose of DXR (90 mg/kg bw) was selected on the basis of its effectiveness in inducing chromosomal aberrations in rodents [9]. The doses of RES (6.25, 12.5 and 25 mg/kg bw) were selected on the basis of preliminary experiments. The administration of ethanol plus distilled water (1:9) did not induce any significant change in the parameters investigated, when compared to controls that received only distilled water. In the definitive experiments, rats were divided into six groups with six animals each: one control group (distilled water only i.p.), two RES-only groups (12.5 or 25 mg/kg bw i.p.), two DXR-RES groups (90 mg/kg bw + 12.5 or 25 mg/kg bw i.p.), and one DXR group (90 mg/kg bw i.p.). For all groups receiving RES, the following administration schedule was used: the first dose was given 30 min before the time of DXR administration, the second dose concurrently with DXR, and the remaining doses every 6 h thereafter, for a total of five RES doses. The DXR and DXR-RES groups received a single dose of doxorubicin. Animals were sacrificed by cervical dislocation 24 h after the DXR dose. Bone marrow was harvested and processed.

2.3. Preparation of the rat bone marrow cell system

Bone marrow cell preparations for the analysis of chromosomal aberrations were produced by the colchicine-hypotonic citrate technique [37]. All animals were injected i.p. with 2.0 mM colchicine (CAS C9754, Sigma Aldrich, Istanbul, Turkey) 75 min before sacrifice, 24 h after doxorubicin administration. Bone marrow cells were collected from femurs by flushing in warm (37 °C) potassium chloride (0.075 M) solution with a hypodermic syringe, aspirated and incubated at 37 °C for 25 min. The material was then centrifuged (1000 rpm for 10 min). After removal of the supernatant with a Pasteur pipette, fixative (acetic acid/methanol (1:3)) was added. After 25 min, the material was centrifuged (1000 rpm for 10 min). This wash process (centrifuge/resuspend in fixative) was repeated three times. The final cell suspension was dropped onto a chemically-cleaned slide, air-dried, and stained for 7 min with 8% Giemsa stain (8 ml of stock stain + 90 ml distilled water + 2 ml phosphate buffer, pH 6.8), washed, and mounted in Entellan (Merck). Slides were coded before scoring.

Chromosomal aberrations in the cells were scored on a well-spread metaphase plate with 42 ± 1 chromosomes. Per animal, 100 metaphases were analyzed blind, to determine the frequency of chromosomal aberrations. The mitotic index was obtained by counting the number of mitotic cells in 1000 cells/animal. The chromosome aberrations were classified according to Savage's classification [38].

2.4. Statistical analysis

Statistical analyses of the differences in frequencies of chromosome aberrations, abnormal metaphases, and mitotic index between groups were obtained by a one-way ANOVA test. Because variances between groups were unequal, the mean values of each group were compared by the Mann–Whitney test. The mean ± SD were determined for each group. Probability $p < 0.05$ was considered to be significant. Gaps were counted but not included in the statistical analysis, since their cytogenetic significance is not well established [9].

3. Results

The results of the rat bone marrow chromosome aberration analysis following treatment with RES alone, DXR alone, and DXR + RES are presented in Table 1. Based on Savage's classification [38], six structural chromosome aberrations were identified in the control and experimental groups. Treatment with RES (12.5 or 25.0 mg/kg bw) alone did not induce a significant increase in either the frequency of chromosome aberrations or the number of abnormal metaphases, as compared with control values. This demonstrates that RES, at these doses, was not clastogenic in vivo in rat bone marrow cells.

Animals treated with a single dose of DXR (90 mg/kg bw) showed an increased frequency of all chromosome aberration types, total chromosome aberrations, and abnormal metaphases compared to the control group ($p < 0.05$). In the DXR group, chromatid-type breaks were observed at the highest frequency, followed by complex exchanges, triradial figures, quadryradial figures, and isochromatid breaks.

Repeated administration of RES at 12.5 mg/kg in the DXR + RES 12.5 group reduced the frequency of total chromosome aberrations and abnormal metaphases induced by DXR, but the effect was not statistically significant. However, a significant reduction was observed in the frequency of chromatid breaks ($p < 0.05$). In the DXR + RES 25 group, animals administered RES at 25 mg/kg had a statistically significant reduction in total chromosome aberrations and abnormal metaphases induced by DXR when compared to animals treated with DXR alone ($p < 0.05$). RES 25 mg/kg significantly reduced DXR-induced chromatid breaks and quadriradial figures compared to the DXR group but did not reduce isochromatid breaks, complex exchanges and triradial figures.

A significant difference in mitotic index values was not observed between the control group and RES 12.5 or 25. The animals treated with a single dose of DXR showed a significantly lower mitotic index compared to the controls ($p < 0.05$) (Table 1). The rats in DXR + RES 12.5 group showed no significant differences in mean mitotic index