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In vitro tests of resveratrol radiomodifying effect on rhabdomyosarcoma cells by comet assay

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ABSTRACT

Cancer is a global public health problem. Resveratrol is a defensive polyphenol that is synthesized by a wide variety of plants in response to exposure to ultraviolet radiation or also due to mechanical stress caused by the action of pathogens and chemical and physical agents. Grape vines have a high capacity to produce resveratrol, so grape juice and wine, mainly red wine, are considered good sources of resveratrol. The protective effects of resveratrol include promotion of antiinflammatory response, antitumor activity and prevention of degenerative diseases, reduced incidence of cardiovascular diseases and inhibition of platelet aggregation, among others. Therefore, resveratrol is considered to be a cell protector. However, at high concentrations, resveratrol promotes contrary effects by sensitizing cells. The aim of this study was to investigate *in vitro* the radiomodifying effect of resveratrol in culture of human rhabdomyosarcoma cells (RD) by applying the comet assay to evaluate the cell damage and repair capacity. The LD_{50} (lethal dose) obtained was 499.95 ± 9.83 Gy (Mean ± SD) and the CI_{50} (cytotoxicity index) was 150 μM in the RD cells. Based on these data, it was defined the gamma radiation doses (50 and 100 Gy) and resveratrol concentrations (15, 30 and 60 μM) to be used in this study. The results indicated that resveratrol acts as a cell protector at a concentration of 15 μM and has a cytotoxic effect at 60 μM. However, with the interaction of the gamma radiation, the concentration of 60 μM did not produce a statistically significant radiosensitizing effect.

1. Introduction

Contrary to widespread popular belief, cancer is a disease that has existed for millions of years. Fossil dinosaur bones have been found with clear evidence of tumors. Archeologists found a 2700-year-old human skeleton showing evidence of metastasis from prostate cancer. The Greek physician Hippocrates used the word "crab" to describe the disease, because the tumor and its network of blood vessels that resembles crustacean, with its body and legs (Dunn, 2012).

Cancer was a relatively rare disease in ancient times. However, for the past two centuries or so, the incidence of cancer in the population has been growing drastically. This increase is due to two factors: the increasing average human lifespan and the greater exposure to carcinogenic chemical products and X-rays, such as during airplane trips at high altitude (David and Zimmerman, 2010).

According to Brazil's National Cancer Institute (Instituto Nacional do Câncer, 2012), cancer is a growing global public health problem. Forecasts are for some 518,000 cases of cancer in Brazil in 2013.

According to Murad and Katz (1996), rhabdomyosarcoma is the most common sarcoma in infancy. Although it is rare, it demonstrates a serious problem because its correct treatment is difficult. Rhabdomyosarcomas are very resistant to radiation therapy, so they require high doses. They grow from the embryonic mesenchyme, or mesodermal tissue, and can appear in any anatomical area, but most commonly occur in the genitourinary region (21%), extremities (20%), parameningeal region (nasopharynx, nasal cavity, paranasal sinuses, middle ear and infratemporal fossa) (14%), other sites on the head and neck excluding orbits (13%) and only the orbits (10%).

In the nature chemical compounds exist that are capable of inhibiting carcinogenesis at various stages, among them resveratrol (3,4′,5-trihydroxy-trans-stilbene), which is a polyphenol belonging to the set of compounds called phytoalexins (Jeandet et al., 2002). This defensive polyphenol is synthesized by a wide range of plants in response to exposure to ultraviolet (UV)

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radiation or mechanical stress caused by the action of pathogens or chemical or physical agents (Van Etten et al., 1994).

Grape vines have a high capacity to produce resveratrol (Minireview, 2000). As a result, the concentration of resveratrol in wine, especially red wine, is relatively high. For non-drinkers, grape juice, especially the dark variety, is also considered an excellent source of resveratrol, although the concentration is lower than in red wine (Sautter et al., 2005).

The protective effects exercised by resveratrol against damage to the cellular genetic material, produced by the oxidative effect of free radicals, promotes reactions such as induction of the antiinflammatory response (Leonard et al., 2003); antitumor activity (Minireview, 2000); prevention or inhibition of degenerative diseases (Vingtdeux et al., 2008); reduced incidence of cardiovascular diseases (Goldberg et al., 1995); inhibition of platelet aggregation (Minireview, 2000), and inhibition or reduced incidence of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Vasanthi et al., 2012).

A chemical compound such as resveratrol that acts both as a protector and sensitizer of cells is defined as a radiomodifying compound. At low concentrations, resveratrol has a protective effect in cells, but at high concentrations it sensitizes cells (Caddeo et al., 2008; Stocco et al., 2012; Mukherjee et al., 2010).

Red wines and white wines that contain resveratrol also possess cardioprotective properties at low to moderate doses (10–15 μM) (Mukherjee et al., 2010).

In case of human cancer cells (HT29, SW-620, HT-1080) and endothelial cells, resveratrol enhances proliferation when used at low dose (0.1–1 g/mL) and induces apoptosis and decreases mitotic activity when used at high dose (10–100 g/Ml) (Mukherjee et al., 2010).

One study from France shows that at high dose (60 μM), resveratrol inhibits the growth and induces apoptosis in case of both normal (60 μM) and leukemic (5–43 μM) hematopoietic cells (Ferry-Dumazet et al., 2002).

Caddeo et al. (2008) showed that the effect of resveratrol (incorporated in liposomes) on the cell proliferation, cell viability of HEK 293 cell before and after UV-B irradiation is dose dependent. They showed that at low concentration, resveratrolincreased the number of cells without any evidence of toxicity. In contrast, cells treated with higher concentration of resveratrol showed loss of membrane integrity.

Moreno et al. (2012) showed that cell viability decreases with increasing gamma radiation dose. When the radiation dose is close to gamma radiation LD, there is no change of cell viability in relation to resveratrol concentration. However, when gamma radiation is within 500 and 800 Gy it is observed a cell viability increase in the presence of 25 and 30 μM resveratrol concentrations. The results suggest that resveratrol has radioprotective effect in normal cells (NCTC-L929) culture at 25 μM and 30 μM resveratrol concentrations when irradiated at 500 Gy and 800 Gy doses (higher than LD = 354 Gy).

Ionizing radiation interacts with the living organism by direct reactions on biological molecules, as well as by indirect reactions through generation of free radicals and reactive oxygen species that can attack various molecules contained inside cells, such as DNA, protein, lipids and carbohydrates. Water is the substance with the greatest probability of interacting with ionizing radiation, since it accounts for up to 85% of the cell content (Down and Tilson, 1999; Mettler and Upon, 1995; Getoff, 1996). Various DNA lesions can be produced by radiation, direct or indirectly, such as single-or-double-strand breaks, base damage, and DNA–DNA or DNA–protein cross-links.

Various methods have been developed and improved to detect and quantify damages to DNA induced by ionizing radiation. Among them is the biochemical technique known as the comet assay, or microgel electrophoresis assay, which was first described by Ostling and Johanson (1984), utilizing neutral conditions for the lysis and electrophoresis of cells, which permits observing double breaks in DNA strands.

Singh et al. (1988) described the alkaline version of the comet assay, which enables detection of breaks both in single and double strands as well as at alkali-labile sites. For this reason, the alkaline technique is more effective in detecting primary radio-induced lesions.

The comet assay detect the lesions occurring in individual cells and for its simplicity and rapid execution, proved to be adequate to study radioinduced DNA repairable damage, oxidative stress, apoptose and cell repair in all mammalian cell (Oliveira et al., 2001; Nascimento et al., 2001).

The aim of this study was to investigate *in vitro* the radiomodifying effect of resveratrol in human rhabdomyosarcoma cells (RD) by applying the comet assay to assess the damage and repair capacity of cells.

2. Materials and methods

2.1. Cell cultures

The RD cell is a human rhabdomyosarcoma cell line obtained from the American Type Culture Collection (ATCC). The cell preparation was carried out at the Cell Culture Unit of the Adolfo Lutz Institute in São Paulo. The RD cells were cultivated in a cell culture bottle with capacity of 25 cm^2 using Eagle + L-15 medium, supplemented with 15% bovine fetal serum (BFS). The cells were kept at 37 °C in a humid atmosphere containing 5% CO_2 until formation of the cell monolayer.

2.2. In vitro determination of the cytotoxicity index (CI_{50}) of resveratrol

The cytotoxicity index of resveratrol in the RD cell culture was determined by the neutral red uptake assay, according to the international standard (International Organization For Standardization, 2009) and the methods described in the literature (Moreno et al., 2012; Rogero et al., 2003; Magalhães et al., 2011).

The resveratrol was obtained from Sunrise Chemical Ltd. (China), imported and sold by the company PharmaNostra (Campinas, São Paulo). The importer attests that the product is the trans-resveratrol isomer form, with 99.4% purity.

The resveratrol was diluted in ethanol p.a., to obtain the stock solution (50 mM), from which five serial dilutions were formulated, corresponding to 100%, 50%, 25%, 12.5% and 6.25%, where 100% = 250 μM.

Natural rubber latex extract was used as positive control in the culture medium and an extract of high-density polyethylene was used as negative control. The choice of natural rubber latex extract and high-density polyethylene as positive and negative controls, respectively, was based on ISO 10993-part 5 (International Organization For Standardization, 2009). The control solutions were diluted to five different concentrations, the same as those of the resveratrol solutions.

The solutions were sterilized with a membrane system containing pores with diameter of 0.22 μm, in environment previously sterilized with UV radiation for 30 min and 70% alcohol.

A volume of 0.2 mL of each solution (positive control, negative control and resveratrol) was added to each well of a 96-well plate containing 5×10^4 RD cells/well and 0.2 mL of culture medium in the control wells. This plate was then incubated at 37 °C in a humid atmosphere containing 5% CO_2 for 24 h.

After incubation, the plate culture medium was replaced by a neutral red stain solution diluted in Eagle + L-15 culture medium without BFS. Next, the plate was incubated for 3 h for uptake of the neutral red dye by the living cells. Then the dye solution was removed and the plate was washed twice with PBS pH 7.4 and once with a cleaning solution ($CaCl_2$ 0.1% in formaldehyde 0.5%). Finally, the extraction solution (2% acetic acid and ethanol, 1:1) was added, causing cell lysis and release of the dye.

The optical density (OD) values of each well in the plate were read by an ELISA spectrophotometer, equipped with a 540 nm filter. The cell viability percentages were calculated from the OD values in relation to cell control and the graphs were plotted using the GraphPad Prism5 program.

2.3. In vitro determination of the 50% lethal dose (LD_{50}) of gamma radiation

The used gamma radiation was from a ^{60}Co GammaCell 200 source (Atomic Energy of Canada Limited – Ottawa, Canada), available at Radiation Technology Center (CTR) of the Nuclear and Energy Research Institute (IPEN-CNEN/SP).

To measure the 50% lethal dose of gamma radiation in the RD cell culture, five 96 wells microplates were used containing 5×10^4 RD cells/well. Each plate was submitted to a different radiation dose: 1000 Gy, 750 Gy, 500 Gy, 250 Gy and 0 Gy (control plate).

After irradiation, the culture medium was exchanged for fresh culture medium and the plates were incubated at 37 °C under a humid atmosphere containing 5% CO_2 for 24 h. After this period, the procedures were the same as described in the preceding item (*In vitro* determination of the cytotoxicity index (CI_{50}) of resveratrol).

2.4. Comet assay (microgel electrophoresis assay)

The alkaline version of the comet assay was used, as described by Singh et al. Singh et al. (1988). One day beforehand, the histological slide was cleaned with ethanol and a film of normal agarose (dissolved in PBS buffer free of Ca and Mg, at 65 °C) was prepared on the top and left at room temperature to dry.

To carry out the comet assay, it was necessary to perform two previous steps: incubation of the RD cells with resveratrol and irradiation of these cells, and finally application of the comet assay.

2.4.1. Incubation of the RD cells with resveratrol

The resveratrol stock solution (50 mM) was used to produce three dilutions (60 μM, 30 μM and 15 μM) in Eagle + L-15 medium supplemented with 15% BFS. A 0 μM vehicle solution was also used in the experiments. For each resveratrol concentration was used two RD cells culture bottles (25 cm^2 volume). The culture medium was replaced by resveratrol solution while the control bottle received only fresh culture medium. These resveratrol solutions stayed in contact with RD cells in the respective culture bottle for 24 h. The bottles were incubated at 37 °C in a humid atmosphere containing 5% CO_2 for 24 h, for subsequent irradiation and analysis of cell damage and repair, by applying the comet assay.

2.4.2. Irradiation of the RD cells

After incubation of the RD cells with resveratrol, the cells in the culture bottles were harvested by trypsinization treatment. The cell suspension was centrifuged and the RD cells were resuspended in PBS without EDTA. The cell suspension was adjusted to 1×10^6 - cells mL^{-1}, and 3 mL of this suspension was placed in 15-mL test tubes and these tubes were irradiated by different doses: 100 Gy, 50 Gy and 0 Gy (control). Immediately afterward, the irradiated tubes were placed in an ice bath. Each test with a different resveratrol concentration was accompanied by a control sample in a tube that was not irradiated.

2.4.3. Application of the comet assay

The comet assay was performed for each resveratrol concentration at 0, 24 and 48 h after irradiation, for all radiation doses (including 0 Gy as control of cell viability).

The first microgel electrophoresis procedure was performed just after irradiation of the cells (time 0). The other samples were processed, plated in culture bottles and incubated at 37 °C in a humid atmosphere containing 5% CO_2 for 24 and 48 h, to evaluate the level of DD in relation to time 0 h. After these two intervals, the cells were harvested by trypsinization and the cell suspension was adjusted and diluted in PBS without EDTA for the corresponding assay.

With the processed cells (0, 24 and 48 h after irradiation), 10 μL of the cell suspension were added to 90 μL of low melting agarose. The mixture was placed on a slide containing a normal agarose layer prepared in advance. After solidification (5 min at 4 °C) the slides were placed in a cell lysis solution (2.5 M of NaCl, 100 mM of EDTA, 10 mM of Tris, 1% sodium sarcosinate, 1% Triton X-100 and 10% DMSO) for 2 h at 4 °C to remove the proteins.

After cell lysis and protein extraction, what remained on the slides were the cell nuclei, named as nucleoids. In this moment, the assay procedures were carried out in dark environment. The slides removed from the lysis solution were placed side-by-side in the tray, containing alkaline electrophoresis buffer (1 mM of EDTA and 300 mM of NaOH, pH 12), for 30 min, to allow expression of the breaks in the strands (single and double) and alkali-labile sites of the DNA.

Then the slides were submitted to electrophoresis (25 V and 300 mA) for 30 min at 4 °C (tray in an ice bath). After the electrophoreses, the slides were neutralized three times for 5 min with Tris buffer (pH 7.5) and fixed in 100% ethanol for 10 min, after which they were stored for subsequent analysis.

The slides were stained with 50 μL of ethidium bromide (20 μg mL^{-1}) for analysis under a fluorescence microscope (Carl Zeiss) with 200× magnification. About 100 randomly chosen comets were analyzed on each slide.

The comets were classified by the intensity of fragmentation of the nucleoids into five categories (0–IV), according to the criterion established by Jaloszynski et al. (1997) and Mozdarani et al. (2007). Comets were classified as in class 0 that had bright head and no tails (no DNA migration), while the comets with small heads and long and/or diffuse tails were classified as category IV and those with intermediate aspects were classified in classes I, II and III.

The estimated quantity of DNA damage was determined according to the equation described by Jaloszynski et al. (1997), varying from 0 to 400 arbitrary units (au), as described below:

$$DD = (n1 + 2n2 + 3n3 + 4n4)/(\Sigma/100) \quad (1)$$

where DD is the damage to the DNA in arbitrary unit (au), $n1$–$n4$ are number of comets in classes I–IV and Σ is total number of comets analyzed, including class 0.

The DD (DNA damage) values can indicate various situations, from all undamaged cells (class 0) (0 au) to all highly damaged cells (class IV) (400 au). The graphs for analysis of the DD values were plotted using the GraphPad Prism5 program. The statistical analysis was by two-way ANOVA and the Bonferroni post-test, with $p > 0.05$ considered not statistically significant.

3. Results and discussion

Based on the results of gamma radiation LD_{50} and the resveratrol CI_{50} for RD cell line, it was used two radiation doses (50 Gy

and 100 Gy) and three resveratrol concentrations (15 μM, 30 μM and 60 μM) for the *in vitro* study of the radiomodifying effects of this compound. The results were analyzed in relation to the control, called "NT" (not treated with resveratrol).

3.1. In vitro determination of the cytotoxicity index (CI_{50}) of resveratrol

In this test, the OD values obtained were used to calculate the cell viability percentages in relation to the control cells, considered to be 100% viable. Fig. 1 shows the cell viability curves in function of extract concentration for the negative and positive controls and resveratrol.

The biological assay to determine the CI_{50} of resveratrol in tumor-line RD cell culture was performed to determine the concentrations to be used in the comet assay.

In a study of the same cell line (RD), Chow et al. (2005) obtained a CI_{50} for resveratrol of 48 μM, while the corresponding value found in this study was 150 μM. The difference may have been due to the cell density and cytotoxicity assay method used. Although the number of cells used in both studies was equal (5×10^4 cells per well), the cell density was different, because we used a 96 wells microplate with growth area of 0.31 cm^2, while Chow et al. (2005) used a 24 wells microplate with a growth area of 1.91 cm^2. Also, in the method used by Chow et al. (2005), the RD cells remained in contact with the resveratrol for three days, after which the solution was exchanged for fresh culture medium free of resveratrol and the cells were incubated for a further 48 h which could have promoted cell proliferation. In contrast, in our study the cells were incubated with resveratrol for 24 h, and after that the assay was finished.

The CI_{50} is obtained by the intersection of the cell viability curve and the 50% cell viability line. The CI_{50} indicates the concentration in which a compound reduces the cell population by 50%. For the tumor line RD cells, the resveratrol CI_{50} was 150 μM (Fig. 2).

3.2. In vitro determination of the 50% lethal dose (LD_{50}) of gamma radiation

With the mean OD of each 96 wells microplate, we calculated the cell viability percentage obtained at each radiation dose in comparison with the control plate (0 Gy), considered to have 100% viability.

The LD_{50} is the radiation dose that causes 50% cell death, obtained graphically in the intersection of the curve with the 50% viability line. The value found for the tumor RD cells line was 500 Gy (Fig. 3). It was not found any reference in the literature about ionizing radiation LD_{50} to the tumor line RD cells.

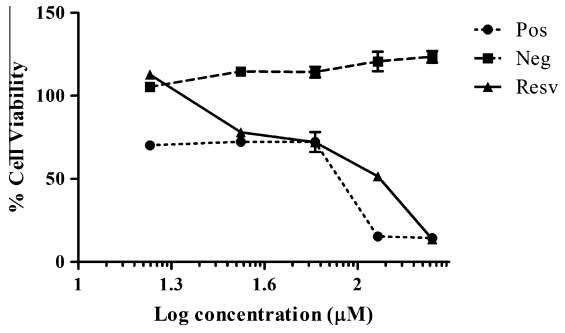

Fig. 1. Cell viability curves obtained in the *in vitro* cytotoxicity test of resveratrol, by the neutral red uptake assay. *Pos:* Positive Control; *Neg:* Negative Control; *Resv:* Resveratrol. Points represent the mean ± SEM of three separate experiments ($n = 3$).

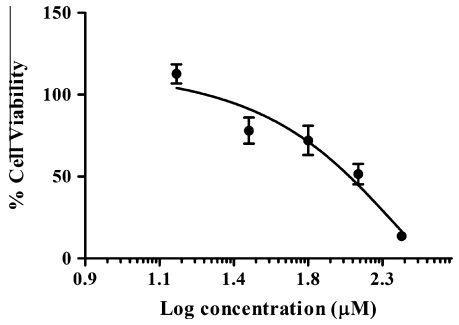

Fig. 2. Dose–response curve of RD cultures treated with serial dilutions (0–250 μM) of purified resveratrol. Solid line represent curve fit to dose–response sigmoidal model (R^2: 0.909). Each data point represents the mean ± SEM of three independent experiments ($n = 3$).

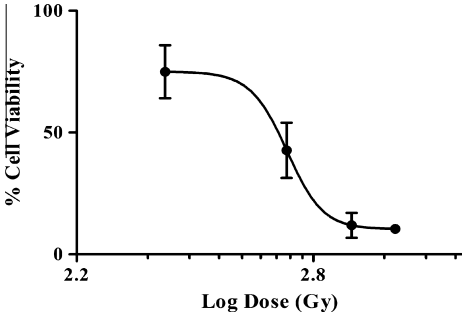

Fig. 3. Dose–response curve of RD cultures irradiated at increasing doses of ionizing radiation (0–1000 Gy). Solid line represent curve fit to dose–response sigmoidal model (R^2: 0.911). Each data point represents the mean ± SEM of three separate experiments ($n = 3$).

The biological assay to determine the lethal dose of gamma radiation on the tumor line RD cells was carried out to define the radiation doses to be used in the comet assay.

3.3. Comet assay

The comet assay was performed to determine *in vitro* the radiomodifying effects of resveratrol. This test allows analyzing both the damage produced by the action of gamma radiation on tumor cells and the possible DNA damage reduction in the presence or not of resveratrol, as well as the damage induced by high concentrations of resveratrol in the absence of gamma radiation. For analysis of the DD, the assays were carried out just after irradiation of the cells, while for analysis of possible cell repair the assays were performed 24 and 48 h after irradiation.

The results of classified comets number obtained by visually counting and classifying the comets (Fig. 4) were applied in Eq. (1) to calculate the DD values.

Table 1 shows the DD values of non-irradiated RD cells at different resveratrol concentrations (0 μM, 15 μM, 30 μM and 60 μM) and analyzed at different times (0 h, 24 h and 48 h).

Fig. 5 shows the damage to the DNA induced by the different concentrations of resveratrol (0 μM, 15 μM, 30 μM and 60 μM), analyzed at different times (0 h, 24 h and 48 h) after administration.

According to other authors (Stocco et al., 2012; Penumathasa and Maulik, 2009; Xi et al., 2009), a resveratrol concentration of 15 μM is considered to exercise *in vitro* a protective effect, while concentrations above 15 μM induce apoptosis in tumor cells. Further according to the literature, 60 μM is within the concentration range of resveratrol known to inhibit the metabolic activity and

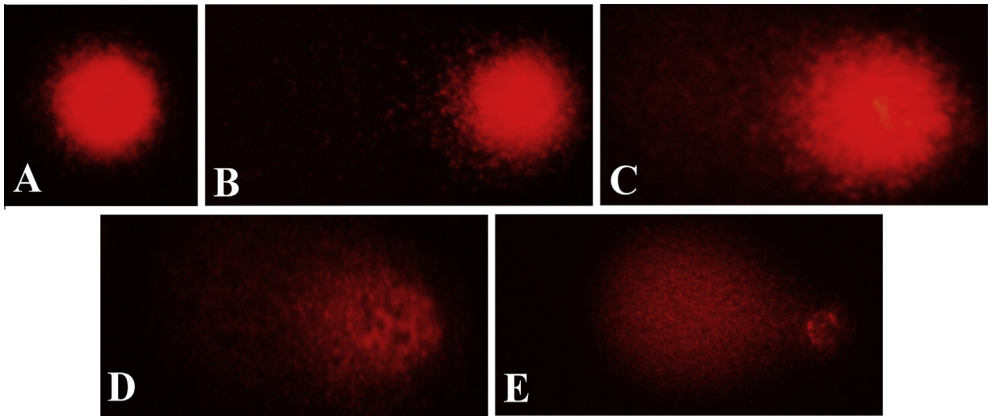

Fig. 4. Micrographs of RD cells, with visual classifications of the comets: (A) comet class 0; (B) comet class I; (C) comet class II; (D) comet class III and (E) comet class IV. Events were visualized under 10 × magnification.

Table 1
DD values for the RD cells not irradiated (0 Gy), at different resveratrol concentrations.

Resveratrol concentration (μM)	DD (au) Evaluation time		
	0 h	24 h	48 h
0	35 ± 11	30 ± 12	26 ± 7
15	20 ± 2	13 ± 6	11 ± 5
30	19 ± 5	19 ± 7	13 ± 4
60	45 ± 33	56 ± 29	48 ± 30

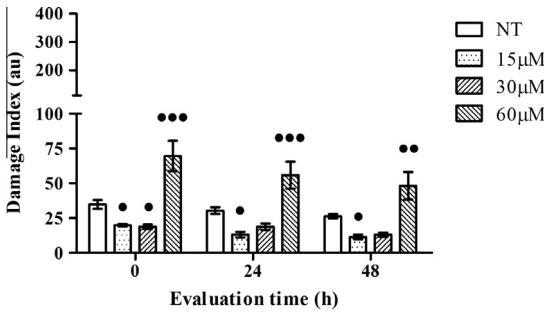

Fig. 5. Control comet assay of the RD cells not exposed to radiation: DNA damage (DD) after incubation with resveratrol at different concentrations and analyzed at different times: • $p < 0.05$; •• $p < 0.01$; ••• $p < 0.001$. Each data point represents the mean ± SEM of three separate experiments ($n = 3$).

cell proliferation (Caddeo et al., 2008; Stocco et al., 2012; Chow et al., 2005; Hope et al., 2008). We chose the concentration of 30 μM as intermediate between the concentrations that induce radioprotective and radiosensitizing.

In the samples not submitted to radiation, analyzed immediately (0 h), resveratrol at the concentration of 60 μM induced a statistically significant increase in the DD values, evidencing the intrinsic cytotoxicity of resveratrol. The other concentrations (15 μM and 30 μM) significantly diminished the DD values, showing the protective effect exercised by resveratrol on the RD cells. At 24 h and 48 h, a possible cell repair was noted at all the resveratrol concentrations, as well as in the control cells. Comparing the possible cell repair capacity of 60 μM resveratrol with control and with the other concentrations showed no statistically significant.

Table 2 shows the DD values of the RD cells irradiated with 50 Gy and 100 Gy obtained 0, 24 and 48 h after irradiation, at different resveratrol concentrations.

The comet assay graphs (Figs. 6 and 7) show the DNA damage in relation to resveratrol concentrations (0 μM, 15 μM, 30 μM and 60 μM) analyzed at different times (0 h, 24 h and 48 h) after irradiation at doses of 50 Gy and 100 Gy.

The data on the RD cells irradiated at the dose of 50 Gy, in the presence of different resveratrol concentrations, showed that only the resveratrol concentration of 15 μM ($p < 0.001$) showed significantly DD value reduction of RD cells, 48 h after irradiation, indicating its radioprotective property. The other resveratrol concentrations did not have statistically significant effects by the used method (Fig. 6).

RD cells in the presence of 30 μM resveratrol and irradiated at 100 Gy, showed a small decline ($p < 0.05$) in the DD value soon after irradiation (time = 0 h). Twenty four hour after irradiation, a substantial and significant reduction of the DD values was observed at 15 μM ($p < 0.001$) and 30 μM ($p < 0.001$) resveratrol concentration, probably due to repair process suggesting radioprotective effect. This process continued 48 h after irradiation, especially in the 15 μM ($p < 0.001$) concentration. Resveratrol at 60 μM presented a no statistically significant results, however a

Table 2
DD values for the RD cells irradiated with 50 Gy and 100 Gy, obtained 0, 24 and 48 h after irradiation, at different resveratrol concentrations.

Resveratrol concentration (μM)	DD (au) Evaluation time					
	50 Gy			100 Gy		
	0 h	24 h	48 h	0 h	24 h	48 h
0	302 ± 19	272 ± 17	248 ± 40	336 ± 17	319 ± 14	292 ± 18
15	286 ± 32	253 ± 28	204 ± 63	328 ± 17	281 ± 13	249 ± 29
30	283 ± 16	247 ± 20	236 ± 15	315 ± 13	281 ± 19	269 ± 18
60	301 ± 21	299 ± 17	274 ± 23	329 ± 33	334 ± 8	308 ± 8

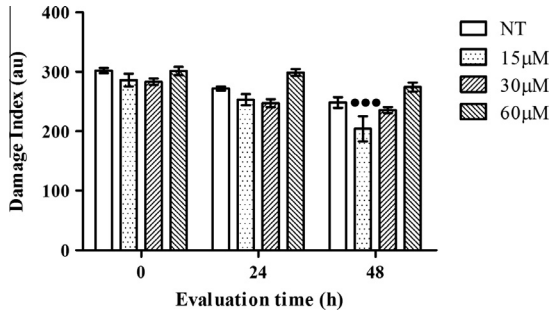

Fig. 6. Comet assay: damage to the DNA of the RD cells after incubation with resveratrol at different concentration and irradiated at a dose of 50 Gy: ••• $p < 0.001$. Each data point represents the mean ± SEM of three independent experiments ($n = 3$).

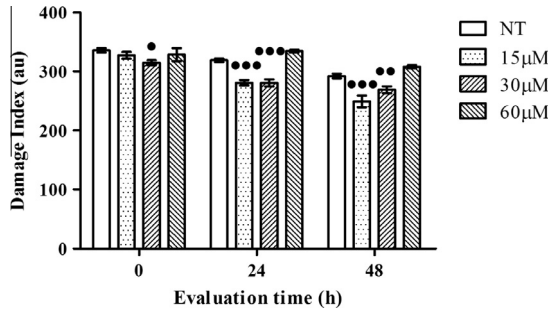

Fig. 7. Comet assay: damage to the DNA of the RD cells after incubation with resveratrol at different concentration and irradiated at a dose of 100 Gy: • $p < 0.05$; •• $p < 0.01$; ••• $p < 0.001$. Each data point represents the mean ± SEM of three independent experiments ($n = 3$).

visually increase in the DD value after 24 h in relation to NT was observed, possibly due to a non-cell repair at 24 h and 48 h. This probably occurred because 60 μM resveratrol prevents or impairs cell repair (Fig. 7).

The data obtained showed a radioprotective effect of resveratrol at the concentrations of 15 μM and 30 μM, but this was only statistically significant at 15 μM, in conformity with other literature results (Stocco et al., 2012; Moreno et al., 2012; Penumathasa and Maulik, 2009; Xi et al., 2009). Resveratrol at 60 μM concentration showed a cytotoxic effect on the tumor cells and appeared to exercise a radiosensitizing effect, but it was not statistically significant. Ferry-Dumazet et al. (2002) showed that a high concentration of resveratrol (60 μM) can inhibit cell growth and induce apoptosis, both in normal and tumor cell lines. According to Chow et al. (2005), 50 μM resveratrol reduces the proliferation of RD cells by around 50%. They also assessed 70 and 100 μM and observed that RD cell proliferation reduces with increasing of resveratrol concentration.

Resveratrol is good for health but the health benefit of resveratrol is dose-dependent. Low doses resveratrol protect health from different types of diseases, while high doses resveratrol can be detrimental for health. However, high dose resveratrol may be required in pathological conditions such as destroying cancer cells (Mukherjee et al., 2010).

According to Heiduschka et al. (2014) resveratrol seems to be a promising agent in combination with radiation therapy due to radiosensitizing effect.

4. Conclusions

The results of this study reaffirm that resveratrol acts as a radiomodifying compound on tumor line RD cells, with a radioprotective effect at low concentrations and radiosensitizing effect at high concentrations. We found that resveratrol acts as cell protector at 15 μM and shows sensitizing effect at 60 μM.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

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