Different responses of vanillic acid, a phenolic compound, in HTC cells: cytotoxicity, antiproliferative activity, and protection from DNA-induced damage

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Genet. Mol. Res. 15 (4): gmr15049388
Received September 28, 2016
Accepted November 11, 2016
Published December 19, 2016
DOI http://dx.doi.org/10.4238/gmr15049388

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ABSTRACT. The consumption of healthy and natural foods has increased over the last few years, primarily because these foods are rich in substances with biological properties of interest, such as exerting anticancer effects and decreasing oxidative stress in living tissues. These foods support adequate nutrition, maintain health, and improve quality of life. Vanillic acid (VA) is a phenolic compound used widely in the food industry as a flavoring, preservative, and food additive. VA can be found in various cereals, whole grains, fruits, herbs, green tea, juices, beers, and wines and possesses antioxidant, hepatoprotective, cardioprotective, and antiapoptotic activities. Studying the cytotoxicity as well as the mutagenic and antimutagenic effects of different concentrations of VA in *Rattus norvegicus* hepatoma cells (HTC) can

identify new cellular activities of this substance. Concentrations up to 100 μM VA are not cytotoxic to HTC cells in a MTT [3-(4,5-dimethilthiazol-2-yl)-2,5-diphenil tetrazolium bromide] assay after 96-h exposure; therefore, VA does not compromise mitochondrial activity. Similarly, concentrations up to 500 μM do not compromise plasma membrane integrity. VA at 10 and 50 μM showed no mutagenic/clastogenic effects, as no significant micronuclei induction was observed. VA 10 μM presented no antiproliferative activity and reduced the cytotoxicity induced by benzo[a]pyrene. The antimutagenic activity of 10 μM VA was observed by the simultaneous, pre-, and post-treatments, as the phenolic compound significantly reduced the frequency of micronuclei induced by the mutagen. These results indicate that VA exerts different responses in HTC cells. Low concentrations present no cytotoxic, mutagenic, or antiproliferative effects and protect cells from DNA damage.

Key words: Antimutagenicity; Cell viability; Clastogenicity; Micronuclei; Mutagenicity

INTRODUCTION

Plant secondary metabolites are derived from the products of primary metabolism but are not directly involved in the basic processes of growth and development. Many of these products play important roles in the interaction between plants and the environment, particularly with the biotic environment, where such substances may act as agents that attract pollinators or seed dispersers, defend against natural enemies or parasites, or serve as allelochemicals during competition. However, many of the functions and benefits plants derive from secondary metabolites remain unknown (Kroymann, 2011; Russell and Duthie, 2011).

Vanillic acid (4-hydroxy-3-methoxybenzoic acid; VA) is a phenolic compound produced by secondary metabolism in plants, and is widely used in the food industry as a flavoring, food additive, and preservative, and in perfumery (Figure 1). Its pleasant vanilla aroma is due to its molecular structure, which corresponds to the oxidized form of the aldehyde vanillin (vanilla). VA can be found in many foods, such as rice, wheat, mango, strawberry, sugarcane, herbs and spices, beer, wine, teas, and juices (Zheng and Wang, 2001; Brindzová et al., 2009; Russell et al., 2009; Gitzinger et al., 2012; Jun et al., 2012; Palafox-Carlos et al., 2012; Alves et al., 2016).

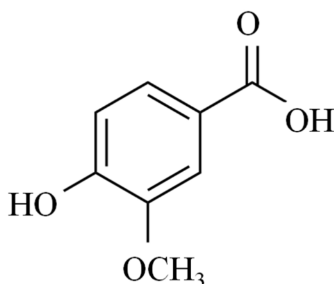

Figure 1. Structure of vanillic acid.

VA exerts strong antioxidant action, exhibits hypotensive, cardioprotective, hepatoprotective, and antiapoptotic activities, inhibits snake venom, and has roles in gene regulation (Dhananjaya et al., 2006; Huang et al., 2008; Dhananjaya et al., 2009; Itoh et al., 2009; Chou et al., 2010; Kim et al., 2010; Kumar et al., 2011; Prince et al., 2011). These biological actions suggest that VA can act as a functional food by contributing to the welfare and health of the population (Navarro et al., 2015) and as a nutraceutical by aiding the treatment and prevention of degenerative diseases, such as atherosclerosis and cancer (Das et al., 2012).

However, studies investigating the cytotoxic activity of VA, its interaction with and ability to change the genetic material of cells (such as the appearance of double-stranded DNA breaks), and its ability to prevent such events are lacking. Only a few studies on these potential roles of VA have been reported in the literature; therefore, we sought to identify the properties of this phenolic compound, which is present even at low concentrations in many natural and processed foods that are consumed daily by human populations. This study determined the cytotoxic effects of VA using the MTT [3-(4,5-dimethilthiazol-2-yl)-2,5-diphenil tetrazolium bromide] assay and investigated the cell viability, mutagenic activity, and antimutagenic activity of VA using the cytokinesis-block micronucleus assay in *Rattus norvegicus* hepatoma cells (HTC) cultured *in vitro*. These cells represent an easy and low cost cultivation system, and possess a metabolism similar to that of human cells.

MATERIAL AND METHODS

Cell lines

HTC cells were provided by the Rio de Janeiro Cell Bank, Brazil (BCRJ catalogue No. 0112). The cells were grown in culture flasks (25 cm^2) with 10 mL DMEM (Dulbecco's Modified Eagle Medium) culture medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen), 0.01% penicillin, and streptomycin (Invitrogen), in a BOD incubator at 37ºC. All treatments were performed in three independent repetitions.

Chemicals

VA (97%; CAS 121-34-6), benzo[a]pyrene (B[a]P; 96%; CAS 50-32-8), doxorubicin (DXR 98%; CAS 25316-40-9), methyl methanesulfonate (MMS; 99%; CAS 66-27-3), cytochalasin-B (CytB; 98%; CAS 14930-96-2), dimethyl sulfoxide (DMSO; 99.7%; CAS 67-68-5), and MTT [3-(4,5-dimethilthiazol-2-yl)-2,5-diphenil tetrazolium bromide; 98%; CAS 298-93-1] were purchased from Sigma (St. Louis, MO, USA). Trypan blue dye was purchased from Invitrogen. VA was diluted in DMEM to a final concentration of 1000 μM. The other concentrations of VA (1, 10, 50, 100, and 500 μM) were prepared by diluting this solution in complete culture medium.

Cytotoxicity assay (MTT)

To detect cytotoxicity, a MTT assay was performed according to the protocol suggested by Mosmann (1983) with modifications. Wells of 24-well cell culture plates were seeded with 5 x 10^4 cells; the control wells contained no cells (blank). The cells were cultured for 24 h with 2 mL DMEM supplemented with 10% fetal bovine serum. After this period, the culture

medium was discarded, and 2 mL fresh medium containing the appropriate agent was added. The following groups were used: negative control (CO-, 50 µL DMEM), cytotoxic agent (MMS, 150 µM), and VA (concentrations of 1, 10, 50, 100, and 500 µM). The blank wells were treated with 500 µM VA. The cells were then incubated for 24, 48, 72, and 96 h following this, the DMEM was replaced with 0.5 mL serum-free medium plus 0.167 mg/mL MTT. The plate was incubated for an additional 4 h; then, the MTT-containing medium was discarded, and each well received 0.3 mL DMSO for formazan crystal solubilization. The absorbance was measured in a microplate reader at 550 nm. The absorbance data were normalized to that of the negative control ($Abs_{treatment}/Abs_{control}$ x 100).

Trypan blue exclusion cell viability assay

Cell viability was assessed on 24-well cell culture plates. In each well, 2.5 x 10^4 cells were seeded in 2 mL complete culture medium plus the following treatments: CO- (50 µL culture medium), cytotoxic agent (DXR, 10 µg/mL), and VA (1, 10, 50, 100, and 500 µM). The cells were incubated for 24 h, trypsinized, and resuspended in 0.5 mL culture medium. An aliquot of the cellular suspension (20 µL) was homogenized with trypan blue (0.4%) dye in a 1:1 ratio. The cells were quantified on a Neubauer chamber hemocytometer. Data are presented as the relative percentage of viability compared to the negative control ($V_{treatment}/V_{control}$ x 100).

Cytokinesis-block micronucleus assay

For the mutagenicity experiment, 1 x 10^6 cells were cultured in 5 mL complete culture medium per flask. After cell-cycle stabilization, the following groups were used: CO- (100 µL culture medium), micronucleus inducer (B[a]P, 10 µg/mL), and VA (10, 50, 100 µM). CytB (3 µg/mL) was added to obtain binucleated cells.

For the antimutagenicity assay, different treatments containing 10 µM VA were used; the treatment lasted for 24 h (simultaneous; SIM) or 48 h (SIM; pre-treatment; PRE; and post-treatment; POST) (Figure 2).

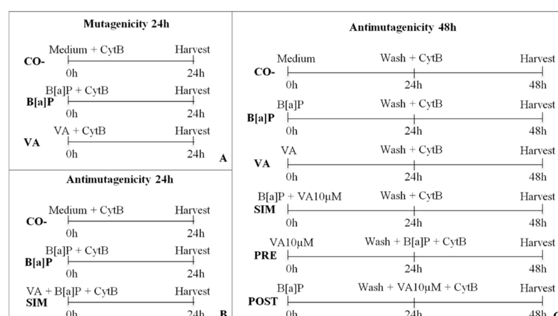

Figure 2. Treatments performed at 24 h (**A.** mutagenesis; **B.** antimutagenesis) and 48 h (**C.** antimutagenesis). 24 h: CO- (negative control: 100 µL culture medium + 3 µg/mL CytB); B[a]P (10 µg/mL + CytB); and SIM (10 µM VA + 10 µg/mL B[a]P + CytB). 48 h: CO- (negative control: 100 µL culture medium); B[a]P (10 µg/mL); VA (10 µM); SIM (10 µM VA + 10 µg/mL B[a]P); PRE (10 µM VA); and POST (10 µg/mL B[a]P). After washing, complete culture medium + CytB was added to all flasks; additional treatments were added to the PRE (10 µg/mL B[a]P) and POST (10 µM VA) groups at 48 h. N = 3.

The cells (1 x 10^6) were seeded in 5 mL complete culture medium per flask, and the treatments were performed after one cell cycle stabilization. In the 48-h treatments, all flasks were washed twice with 5 mL PBS after 24 h, and then, 5 mL complete culture medium and CytB (3 μg/mL) were added for a further 24 h. The PRE group also received B[a]P (10 μg/mL), and the POST group received VA (10 μM).

The cells were harvested after 24 h in steps A and B, and at 48 h in step C to ensure they had completed one or two division cycles under all treatments, respectively, according to the method described by Salvadori et al. (2003). Briefly, the cells were trypsinized, pre-fixed with a drop of formaldehyde, centrifuged (1000 rpm, 5 min), hydrated (1% sodium citrate), centrifuged again, and fixed in methanol-acetic acid (3:1). Slides were prepared by placing one drop of the material on a slide covered with a film of cold distilled water. After drying at room temperature, the slides were stained with Giemsa (5%), dried, and stored in the refrigerator.

One-thousand binucleated cells were counted per replicate to determine the incidence of micronuclei. To determine the cytokinesis-block proliferation index (CBPI), at least 500 cells per repeat were counted; mono-, bi-, and multinucleate cells were differentiated; and the index was calculated with the following formula:

$$\text{CBPI (\%)} = \text{number of mononuclear cells} + \text{bi (x2)} + \text{multi (x3)} / \text{total cells} \quad \text{(Equation 1)}$$

The percentage cytostasis was calculated using the following formula:

$$\text{Cytostasis (\%)} = 100 - 100\,[(\text{CBPI}_{\text{Treatment}} - 1) \div (\text{CBPI}_{\text{Control}} - 1)] \quad \text{(Equation 2)}$$

The percentage damage reduction in the antimutagenicity test was determined by the following formula:

$$\text{Reduction (\%)} = [(\text{Number of cells with MN in A} - \text{Number of cells with MN in B}) \times 100] / (\text{Number of cells with MN in A} - \text{Number of cells with MN in C}) \quad \text{(Equation 3)}$$

where A is the damage-inducing agent; B is the treatment, and C is the negative control.

Statistical analysis

The absorbance data obtained from the MTT assay, the percentage of viable cells in the cell viability test, and the average number of micronuclei were evaluated using ANOVA followed by a Tukey test ($\alpha = 0.05$) with the GrafPad Instat program.

RESULTS

MTT test

The highest VA concentration tested (500 μM) resulted in a statistically significant difference ($P < 0.0001$) in the absorbance value compared to the CO- after 96 h (Figure 3). Treatment with MMS resulted in statistically significant differences at all exposure times ($P < 0.0001$), confirming the responsiveness of the test for substances that interfere with mitochondrial activity.

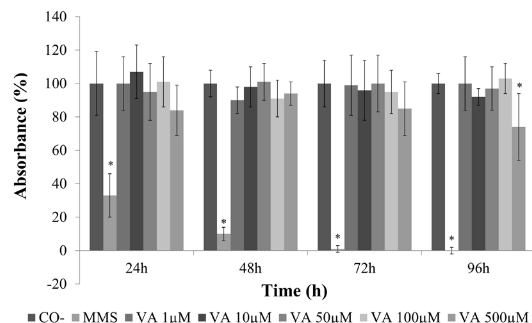

Figure 3. Effect of VA on the activity of mitochondrial succinate dehydrogenase in HTC cells at different times determined by MTT [3-(4,5-dimethilthiazol-2-yl)-2,5-diphenil tetrazolium bromide] assay. The data were normalized (percentage) to the negative control. The values represent the means ± standard deviation; N = 3 wells of 5 x 10^4 cells per well. CO-: negative control (50 µL culture medium); MMS (150 µM); and VA (1, 10, 50, 100, and 500 µM). *Statistically significant difference compared to the negative control (P < 0.0001).

Trypan blue exclusion viability assay

Treatment with VA, at any of the concentrations evaluated, did not significantly affect cell viability as compared with the negative control (Figure 4). The mean cell viability in response to DXR was <80% for the same period of exposure, which was significantly different from that in the CO- (P < 0.0001).

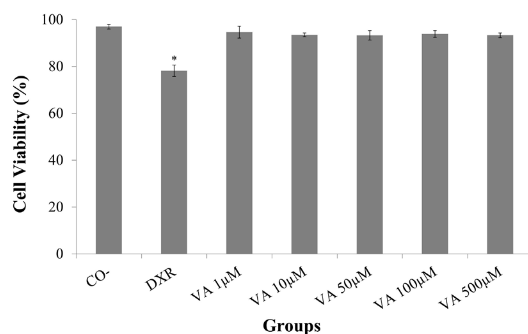

Figure 4. Effect of vanillic acid (VA) on plasma membrane viability of HTC cells after treatment for 24 h. The data are reported as the percentage of live cells. The values represent the means ± standard deviation; N = 3 wells of 2.5 x 10^4 cells per well. CO-: negative control (50 µL culture medium); DXR (10 µg/mL); and VA (1, 10, 50, 100, and 500 µM). *Statistically significant difference compared to the CO- (P < 0.0001).

Cytokinesis-block micronucleus assay

No significant differences were observed in the average number of micronuclei in HTC cells between the 10 and 50 µM VA treatments and the CO-. However, 100 µM VA resulted a significant increment of micronuclei compared with the control (P < 0.001). Furthermore, no significant differences were identified between the B[a]P and 100 µM VA groups (Figure 5).

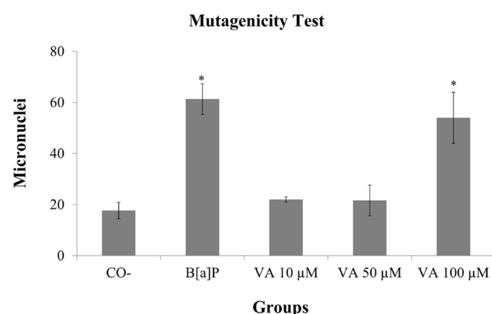

Figure 5. Effect of vanillic acid (VA) on micronuclei induction in HTC cells treated for 24 h. The values represent the means ± standard deviation; N = 3 flasks with 1 x 10^6 cells; 3000 cells analyzed. CO-: negative control (100 µL culture medium); B[a]P (10 µg/mL); and VA (10, 50, 100 µM). *Statistically significant difference compared to CO- ($P < 0.001$).

No statistically significant differences in CBPI were identified between the treatments and the control. A relatively low number of cytostatic cells (5.55%) was observed following treatment with B[a]P compared to the negative control. However, treatment with 10, 50, and 100 µM VA resulted in an increase in the proportion of dividing cells compared with the control (0.15, 6.16, and 4.65%, respectively), as represented by the negative percentage of cytostasis (Table 1).

Table 1. Cytokinesis-block proliferation index (CBPI) and the percentage of cytostasis in HTC cells treated with VA for 24 h.

Groups	Total analyzed cells	Mean CBPI ± SD	Cytostasis (%)
CO-	1500	1.67 ± 0.08	-
B[a]P	1500	1.63 ± 0.04	5.55
VA 10 µM	1500	1.67 ± 0.10	-0.15
VA 50 µM	1500	1.71 ± 0.01	-6.16
VA 100 µM	1500	1.69 ± 0.07	-4.65

CO-: negative control (100 µL culture medium); B[a] P (10 µg/mL); VA (10, 50, 100 µM). N = 3. Negative cytostasis corresponds to the stimulation of proliferation.

A statistically significant difference between the SIM and B[a]P treatments ($P < 0.05$) was observed in the antimutagenicity test (24 h). When the number of cells with micronuclei was considered, the reduction was 57.14%. For the 48-h treatment, no statistically significant difference between the control group and the VA-only treatment was identified. In addition, all VA treatments were able to significantly reduce the frequency of micronucleated cells induced by B[a]P ($P < 0.05$). The greatest reduction in cells with micronuclei occurred with SIM treatment (58.20%), followed by PRE (46.27%), and POST (22.39%) treatment (Figure 6).

No significant differences in CBPI were identified between treatments and the CO- (Table 2) after 24 and 48 h. For the 24-h treatment, SIM treatment caused a statistically significant difference ($P < 0.05$) compared to the negative control, such that the level of cell proliferation increased.

Treatment with B[a]P only for 24 and 48 h led to 16.56 and 16.27% cytostatic cells, respectively (Table 2). The 24-h SIM treatment resulted in negative cytostasis and increased cell proliferation by 18.14%, while the different 48-h treatments reduced proliferation by 9.32% (SIM), 10.74% (PRE), and 7.11% (POST) of cells treated with VA and B[a]P.

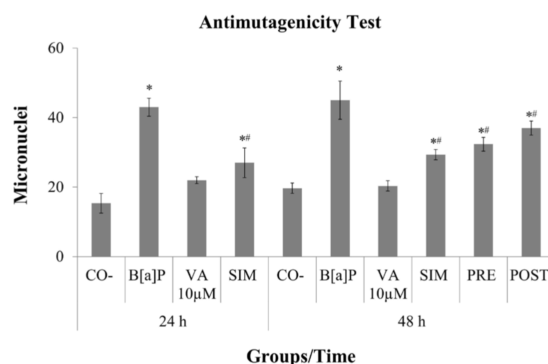

Figure 6. Effect of VA on the reduction of total MN induced by B[a]P in HTC cells with different types and timing of treatments. The values represent the means ± standard deviation; N = 3 flasks with 1 x 10^6 cells; 3000 cells analyzed. 24 h: CO- (negative control: 100 µL culture medium + 3 µg/mL CytB); B[a]P (10 µg/mL + CytB); VA (10 µM); and SIM (10 µM VA + 10 µg/mL B[a]P + CytB). 48 h: CO- (negative control: 100 µL culture medium); B[a]P (10 µg/mL); VA (10 µM); SIM (10 µM VA + 10 µg/mL B[a]P); PRE (10 µM VA); and POST (10 µg/mL B[a]P). After washing, complete culture medium + CytB was added to all wells; additional treatments were added to the PRE (10 µg/mL B[a]P) and POST (10 µM VA) groups at 48 h. *Statistically significant difference compared to CO- ($P < 0.05$). #Statistically significant difference compared to B[a]P ($P < 0.05$).

Table 2. Cytokinesis-block proliferation index (CBPI) and the percentage of cytostasis (compared to the negative control HTC cells) under different types of VA treatment for 24 and 48 h.

Time	Groups	Total analyzed cells	Mean CBPI ± SD	Cytostasis (%)
24 h	CO-	1500	1.63 ± 0,03	-
	B[a]P	1500	1.53 ± 0.02	16.56
	VA 10 µM	1500	1.67 ± 0.10	-0.15
	SIM	1500	1.75 ± 0.06*	-18.14
48 h	CO-	1500	1.63 ± 0.03	-
	B[a]P	1500	1.53 ± 0.09	16.27
	VA 10 µM	1500	1.59 ± 0.01	6.00
	SIM	1500	1.57 ± 0.04	9.32
	PRE	1500	1.56 ± 0.03	10.74
	POST	1500	1.59 ± 0.03	7.11

24 h: CO- (negative control: 100 µL culture medium + 3 µg/mL CytB); B[a]P (10 µg/mL + CytB); VA (10 µM); and SIM (10 µM VA + 10 µg/mL B[a]P + CytB). 48 h: CO- (negative control: 100 µL culture medium); B[a]P (10 µg/mL); VA (10 µM); SIM (10 µM VA + 10 µg/mL B[a]P); PRE (10 µM VA); and POST (10 µg/mL B[a]P). After washing, complete culture medium + CytB was added to all wells; PRE received 10 µg/mL B[a]P, and POST received 10 µM VA, at 48 h. N = 3. Negative cytostasis corresponds to the stimulation of proliferation. *Statistically significant difference compared to CO- ($P < 0.05$).

DISCUSSION

Phenolic compounds are important components of the human diet due to their anticancer properties and their potent antioxidant action in living tissues, which could help to reduce the risk of developing chronic diseases.

Regarding the ability of VA to influence cell viability at the mitochondrial activity level as estimated by the MTT assay, VA was not cytotoxic to HTC cells at any concentration (1, 10, 50, and 100 µM) or after any exposure times (24, 48, 72, and 96 h). The absorbance observed following treatment with 500 µM VA for 96 h was significantly different from that of the CO-, indicating lower mitochondrial activity and, consequently, cytotoxicity in response

to this treatment ($P < 0.0001$). The determination of cytotoxicity is critical for toxicological studies that investigate DNA damage because a reduction in mitochondrial activity may lead to the activation of intrinsic apoptosis pathways, resulting in DNA fragmentation.

Chen et al. (2008) isolated several compounds, including VA, from the medicinal plant *Bulbophyllum odoratissimum* and tested their cytotoxicity by the MTT assay following 72-h exposure in several human cell lines, including leukemia (K562 and HL-60), lung adenocarcinoma (A549), and hepatoma (BEL-7402) cells. Similar to our study, only >600 µM VA reduced the absorbance by 50% compared to the control, indicating low cytotoxicity of this compound. Our results were consistent with those of Chou et al. (2010) who found no cytotoxic effect of 20 or 50 µM VA in murine melanoma cells (B16F0) or human fibroblasts (Hs68) after 72 h of exposure.

The cytotoxicity of VA was also evaluated in the present study using the trypan blue exclusion test. HTC cells treated with VA (1, 10, 50, 100, and 500 µM) for 24 h showed no damage at the plasma membrane level (viability higher than 90%) compared to the CO-. Kanski et al. (2002) also found that 50 µM VA had no cytotoxic effect using rat hippocampus embryonic cells exposed for 24 h.

The level of DNA damage induced by VA was determined using the cytokinesis-block micronucleus assay, which permits the identification of micronuclei in cells that have undergone a nuclear division cycle during exposure to treatment, indicating the mechanism for clastogenicity. Because no cytotoxic activity was observed in HTC cells treated with 10, 50, and 100 µM VA, we chose to use the cells in a mutagenicity test. At concentrations of 10 and 50 µM, VA did not significantly induce micronuclei (22 ± 1 and 21.67 ± 7.37 micronuclei, respectively) compared to the CO- (17.67 ± 3.21). However, 100 µM VA was as effective at inducing micronuclei (54 ± 1.13) as treatment with the mutagen B[a]P (61.33 ± 6.81). These data indicate that even non cytotoxic concentrations of VA, as observed by the MTT and cell viability assays, could be mutagenic/clastogenic and interact with DNA molecules or other related enzymes in HTC cells, inducing a larger number of micronuclei.

Nevertheless, the safety of VA has been questioned by some authors. Erdem et al. (2012) observed that 12 µM VA was genotoxic to human lymphocytes using the micronucleous test and comet assay, while a concentration of 6 µM was not genotoxic. Based on our results, these findings show that VA may induce DNA-damage in hepatoma cells at higher concentrations than in human lymphocytes, probably because of the different responses induced by VA in normal and cancer cell lines. Phenolic compounds should not be considered as pure antioxidants because under certain conditions, they can also display pro-oxidant activity (Erdem et al., 2012). This may have occurred in the present study, resulting in the cytotoxic and mutagenic effects of VA at 500 and 100 µM, respectively. However, Taner et al. (2016) observed no cytotoxicity or genotoxicity in Chinese hamster ovary cells and human lymphocytes at concentrations up to 168 mM VA by the micronucleus test and comet assay.

To investigate the antimutagenicity of VA, we used a concentration of 10 µM because it was not cytotoxic or mutagenic to HTC cells. At 24 h, SIM treatment (cells exposed to both VA and B[a]P) reduced the proportion of induced micronucleated cells by 57.14%. At 48 h, all VA treatments reduced the frequency of micronuclei induced by B[a]P. The greatest reduction in micronucleated cells was observed following SIM treatment (cells exposed simultaneously to VA and B[a]P for 24 h and analyzed after one more cell cycle) (58.20%), followed by PRE (46.27%), and POST (22.39%).

The antimutagenesis mechanisms of VA have also been observed in prokaryotes. Bourgou et al. (2008) used the Ames test and found that the number of revertant bacteria

colonies can be reduced by 70% by treatment with sodium azide and *Nigella sativa* plant extracts, indicating that phenolic compounds, particularly VA, exert antimutagenic activity.

When HTC cells were exposed simultaneously to VA and B[a]P, the number of induced micronuclei decreased. VA may act by reducing the toxicity of the mutagenic compound, reducing the activity of phase I enzymes, which metabolize this inert compound to its active form (benzo[a]pyrene(C)-7,8dihydrodiol-9,10-epoxide), or possibly activating phase II enzymes, which inactivate toxic cellular agents. As cells assimilate VA, the same mechanism may have occurred in the PRE. Similarly, in the POST, the cells were exposed to VA only after damage had been established, resulting in a lower reduction of micronucleated cells. These data also suggest that VA does not affect the activation of the DNA repair system. Appiah-Opong et al. (2007) evaluated the inhibitory effect of VA on five phase I enzymes (CYP3A4, CYP1A2, CYP2B6, CYP2C9, and CYP2D6) and found a slight but significant inhibition (15% on average). This finding reinforces the idea that VA may act by reducing the activity of metabolizing enzymes, which catalyze the conversion of B[a]P in its toxic form, consistent with the results obtained in the present study in response to the SIM and PRE.

Evaluation of CBPI in the mutagenicity test indicated that VA had no cytotoxic effect on the HTC cells with regards to this parameter, and the percentage of cytostasis revealed an increase in proliferating cells under all treatments (0.15, 6.16, and 4.65% for 10, 50, and 100 μM VA, respectively) compared to the CO-. In addition, no cytotoxic effect was observed for CBPI in the antimutagenesis 24-h SIM treatment. B[a]P induced a higher percentage of cytostatic cells (16.56 and 16.27% for 24 and 48 h, respectively), thus reducing cell proliferation. Furthermore, VA reduced this effect (increasing the number of proliferating cells by 18.14% at 24 h and reducing the number of cytostatic cells induced by B[a]P by 9.32, 10.74, and 7.11%, respectively, in the SIM, PRE, and POST at 48 h). These results indicated that VA does not exert a cytotoxic effect at concentrations up to 100 μM, and that at 10 μM, VA reduces cytotoxicity induced by B[a]P in HTC cells. In the antimutagenicity test, VA may have reduced the percentage of apoptotic cells induced by B[a]P, resulting in reduced cytostasis, as evaluated by the CBPI at 48 h. These effects of VA on cell proliferation have not yet been described in the literature.

In conclusion, VA, a phenolic compound consumed by humans mainly through their diet, has no mitochondrial toxicity in HTC cells cultured *in vitro*, which have a metabolism similar to that of human cells, at concentrations ranging from 1 to 100 μM. In addition, no effect on plasma membrane integrity was observed at concentrations ranging from 1 to 500 μM. Furthermore, 10 and 50 μM VA exhibited no mutagenic/clastogenic potential when used in a cytokinesis-block micronucleus assay. Moreover, low concentrations of VA, such as 10 μM, did not present cytotoxic or antiproliferative activities and reduced the level of cytotoxicity induced by B[a]P. Additionally, this low concentration was effective at protecting the DNA from damage by reducing the percentage of micronuclei, demonstrating its marked antimutagenic potential in this assay.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors would like to thank the Coordination for the Improvement of Higher Level Personnel, CAPES, and the Laboratory of Mutagenesis and Environmental Monitoring staff of the State University of Maringá, UEM.

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