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The genotoxicity study of resveratrol in primary gastric adenocarcinoma cell cultures

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Abstract

Gastric cancer is the second most common cause of cancer-related death in the world. Some studies indicate that polyphenolic compounds and antioxidants exert a protective action against gastric cancer. Among the polyphenolic compounds tested and proven effective against gastric cancer is resveratrol, a natural polyphenol present in red wines and various human food items. Resveratrol has been shown to suppress proliferation of a wide variety of tumor cells. We tested the genotoxic activity of resveratrol in primary cell cultures from gastric adenocarcinoma, obtained by mucosal biopsy at upper digestive endoscopy. The adenocarcinoma cells were analyzed for the presence of micronuclei at different concentrations of resveratrol at 48 hours and at 72 hours. The results showed that resveratrol induced micronuclei dose-dependently. The frequency of micronuclei increased progressively with the dose of resveratrol used, the high frequency is in the primary culture initiated from gastric adenocarcinoma: signet ring cell type. The high frequency of micronuclei is at 72 hours at the 20 µg/mL resveratrol and is decreased at low concentrations (5 µg/mL, 10 µg/mL resveratrol). This results show the genotoxic activity of resveratrol in adenocarcinoma gastric cell and the anticancer property of this substance.

Keywords: gastric cancer, adenocarcinoma, resveratrol, cell culture, micronuclei.

Introduction

Gastric cancer is the second most common cause of cancer-related death in the world. Although the incidence of gastric cancer is on the decline, this disease remains a major health problem and a common cause of cancer mortality worldwide, because the disease is usually detected at an advanced stage, and the currently available chemotherapeutic agents are not highly effective. Adenocarcinoma of the stomach constitutes 90-95% of all gastric malignancies and the second most common gastric malignancies are lymphomas. At present, there are limited experimental data regarding specific agents that prevent or retard gastric carcinogenesis. Some studies indicate that polyphenolic compounds and antioxidants exert a protective action against gastric cancer. Among the polyphenolic compounds tested and proven effective against gastric cancer is resveratrol. Resveratrol (3,5,4'-trihydroxystilbene) is a natural polyphenol produced by a wide variety of plants such as grapes (Vitis vinifera), peanuts (Arachis hypogaea), and mulberries in response to stress, injury, ultraviolet (UV) irradiation, and fungal (e.g., Botrytis cinerea) infection [1-3]. The resveratrol

was shown to inhibit the induction, promotion, and progression of experimentally induced cancer [4]. Moreover, resveratrol inhibits transcription and activity of cyclooxygenase-2 [5, 6], an enzyme found to be upregulated in a number of transformed cells and various forms of cancer. Resveratrol has been shown to suppress proliferation of a wide variety of tumor cells, including lymphoid and myeloid cancers, breast, colon, pancreas, stomach, prostate, ovary, liver, lung and cervical cancers, melanoma, and muscles [7]. Resveratrol also exhibits antibacterial effects, including inhibition of growth of different strains of Helicobacter *pylori* [8, 9]. Besides inhibiting proliferation, resveratrol also induces apoptosis either through the caspase-8dependent pathway (receptor-mediated; type I) or the caspase-9-dependent pathway (mitochondrial; type II), or both [7].

A Material and Methods

We tested the resveratrol in primary adenocarcinoma cell culture and we analyzed the frequency of micronuclei at different concentrations of the resveratrol. The adenocarcinoma fragments were obtained by mucosal biopsy at upper digestive endoscopy. Esophagogastroduodenoscopy has a diagnostic accuracy of 95%. This relatively safe and simple procedure provides a permanent color photographic record of the lesion. This procedure is also the primary method to obtain tissue for diagnosis the suspected lesions. Biopsy of any ulcerated lesion should require at least six biopsies taken from around the lesion because of variable malignant transformation.

The histopathological examination was made in the Department of Pathology, Emergency County Hospital of Craiova. The pieces were processed in Pathology Lab of the same hospital. All specimens were fixed in 15% buffered neutral pH formaldehyde and paraffin embedded. Histological sections were stained using current techniques: Hematoxylin–Eosin, trichromic van Gieson and Giemsa (for *Helicobacter pylori*). We used Laurén histological classification with two main types of gastric carcinoma: type I (intestinal) or type II (diffuse). Adenocarcinoma of the stomach is subclassified according to histological description as follows: tubular, papillary, mucinous, or signet-ring cells and undifferentiated lesions.

The gastric adenocarcinoma fragments obtained by gastric biopsy in upper digestive endoscopy were quickly transferred to a cell culture laboratory in RPMI 1640 that contain antibiotics. The adenocarcinoma tumor fragments were minced into very small pieces with sterile scissors. The tumor cells were carefully harvest after washing with phosphate buffered saline (PBS), centrifugation, and were cultivated into a sterile culture flasks 25 cm² containing RPMI 1640 medium (Biochrom AG) supplemented with 10% heat inactivated fetal bovine serum and antibiotic. The flasks were maintained in a humidified incubator at 37°C in an atmosphere of 5% CO2. Isolation of cancer cells was performed by trypsinization when heavy tumor-cell growth is observed. The cell cultures were examined at an inverted microscope (Carl Zeiss Citoval) to approximately 12-24 hours, the success of every culture is cell proliferation and the appearance of normal culture medium. The resveratrol (Sigma Aldrich) diluted in DMSO (dimethyl-sulfoxide) was administrated in adenocarcinoma cell cultures, in different concentrations (5 µg/mL, 10 µg/mL, and 20 µg/mL) at each passage and maintained for 48 hours and 72 hours. In the control culture was administered DMSO without resveratrol.

After 48 hours, and respectively 72 hours, we performed the standard cell preparation and staining with 10% Giemsa stain, and the microscopic slides were examined with an optical microscope Nikon Eclipse E200, images were taken with a digital camera (Canon A650). A total of 1000 tumoral cell were scored per slide at different resveratrol doses.

Results

The initiation of primary cell cultures was made from a case with advanced Borrmann type I gastric cancer (protruded, protrusive) located on the gastric fornix (Figure 1), which histopathologically was gastric adenocarcinoma: signet ring cell type (Figure 2).



Figure 1 – Advanced Borrmann type I gastric cancer (protruded, protrusive) located on the gastric fornix.



Figure 2 – Gastric adenocarcinoma: signet ring cell type.

This case was a 69-year-old male, admitted in the IInd Medical Clinic of the Emergency County Hospital of Craiova for upper abdominal discomfort. Endoscopic examination revealed an advanced Borrmann type I gastric cancer (protrusive). Distal gastrectomy with sufficient duodenal resection was performed. The patient was infected with *Helicobacter pylori*.

Also, another primary cell culture was made from advanced Borrmann type III gastric cancer (ulceroinfiltrative) (Figure 3), which histopathologically was gastric adenocarcinoma papillary type (Figure 4). This case was a case of a 76-year-old woman.

We scored a total 1000 tumoral cells per concentration of resveratrol to emphasize the presence of micronuclei from each culture. As negative control, we used a cell culture treated with solution of DMSO.



Figure 3 – Advanced Borrmann type III gastric cancer (ulcero-infiltrative).



Figure 4 – Gastric adenocarcinoma: papillary type.

An increased frequency of micronuclei in cell culture treated with resveratrol in comparison with the control cell culture indicates that the substance tested induces chromosomal damage in gastric adenocarcinoma cell culture.

In gastric adenocarcinoma cell culture signet ring cell type, the number of micronuclei depends on the exposure time and the resveratrol dose: in the control culture the cells number with one micronucleus is 28 at 48 hours (Figure 5), and at 72 hours it is 32; at the following doses of resveratrol: 5, 10, and 20 μ g/mL the incidence of micronuclei increases progressively (Table 1).

Figure 5 – Cell with micronucleus (from gastric adenocarcinoma signet ring cell type).



 Table 1 – The frequency of micronuclei in adenocarcinoma cell culture signet ring cell type

Cell culture	Cells w micronucl cel	ith one leus/1000 Ils	Cells with two micronuclei/1000 cells	
	48 hours	72 hours	48 hours	72 hours
Control culture	28	32	_	_
Cell culture with 5 μg/mL resveratrol	34	44	2	2
Cell culture with 10 µg/mL resveratrol	40	66	-	2
Cell culture with 20 µg/mL resveratrol	90	122	7	10

At 48 hours and 72 hours, the frequency of micronuclei is increased compared with control culture. The frequency of micronuclei increases progressively with the dose of resveratrol used, the high frequency is at 10 and 20 μ g/mL resveratrol (Figure 6) at 72 hours, and the low frequency is at 48 hours at 5 μ g/mL resveratrol.



Figure 6 – The incidence of micronuclei in gastric adenocarcinoma signet ring cell type at different resveratrol doses, at 48 hours and 72 hours.

Also, we scored the cell with two micronuclei and we observed that at 20 μ g/mL, resveratrol induces this kind of cells and at 72 hours the number of cell with two micronuclei is 10 compared with the control where there are not cells with two or more micronuclei.

In gastric adenocarcinoma cell culture papillary type also the number of cells with one micronucleus increases as follows:

• in control cell culture, it is 18 at 48 hours and 26 at 72 hours;

• in the culture treated with 5 μ g/mL resveratrol, the number of cells with one micronucleus is 34 at 48 hours and 32 at 72 hours;

• in the culture with 10 μ g/mL resveratrol, it is 48 at 48 hours and 66 at 72 hours (Table 2).

Table 2 – The	frequency	of	micronuclei	in	adeno-			
carcinoma cell culture papillary type								

Cell culture	Cells w micronuc ce	vith one leus/1000 ells	Cells with two micronuclei/1000 cells	
	48 hours	72 hours	48 hours	72 hours
Control culture	18	26	-	_
Cell culture with 5 μg/mL resveratrol	34	32	1	2
Cell culture with 10 µg/mL resveratrol	48	66	-	2
Cell culture with 20 µg/mL resveratrol	81	92	4	7

The high frequency is at 72 hours at 20 μ g/mL resveratrol (Figure 7).



Figure 7 – The incidence of micronuclei in gastric adenocarcinoma papillary type at different resveratrol doses, at 48 hours and 72 hours.

The number of micronuclei is slowly increased in gastric adenocarcinoma cell culture signet ring cell type compared with adenocarcinoma cell culture papillary type.

Genotoxic activity of resveratrol is indicated by an increased incidence of micronucleated adenocarcinoma

cells for the treatment group compared with the concurrent control group. In our experiments, the resveratrol induces micronuclei in adenocarcinoma gastric cell culture dose-dependently, and the high incidence of micronuclei is at 20 μ g/mL resveratrol. Micronuclei are formed because of chromosomal breakage or spindle damage. Fragments of whole chromosomes may not be included in the nuclei of the daughter cells following cell division and form single, or multiple micronuclei in the cytoplasm of these cells. These results show the genotoxic activity of resveratrol in adenocarcinoma gastric cell and show the anticancer property of this substance.

Discussion

Resveratrol induced micronuclei in other cell cultures. Schmitt E *et al.* investigated the genotoxic potential of resveratrol. In L5178Y mouse lymphoma cells, they found a reduction in cell proliferation and in cell viability, as well as an induction of micronuclei [10].

In the Chinese hamster lung, the resveratrol induced micronuclei, polynuclei and karyorrhectic cells after a 48 hours treatment and sister chromatid exchanges in a dose dependent manner at concentrations up to 10 μ g/mL. A slight increase in micronuclei occurred with the 24 hours treatment and a dose-dependent increase in micronuclei, polynuclei, and karyorrhectic cells occurred with the 48 hours treatment up to the 10 μ g/mL dose. The resveratrol caused S-phase arrest and induced apoptosis after a 48 hours treatment [11].

Several compounds show structural similarities to *trans*-resveratrol. In mouse L5178Y lymphoma-cells, resorcinol induced trifluorothymidine resistance in the absence of S9. With and without S9, resorcinol induced sister chromatid exchanges in Chinese hamster ovary (CHO) cells, and only with S9 did it induce chromosomal aberrations. Positive results were obtained in the micronulei test [12].

Resveratrol has been shown to suppress proliferation of gastric cancer cells [13-15]. Atten MJ et al. reported that resveratrol inhibited proliferation of nitrosaminestimulated human gastric adenocarcinoma KATO-III and RF-1 cells [13]. It arrested KATO-III cells in the G0/G1 phase of the cell cycle and eventually induced apoptotic cell death by utilizing a protein-kinase C (PKC)-mediated mechanism to deactivate these gastric adenocarcinoma cells. Holian O et al. demonstrated that, in gastric adenocarcinoma cell line SNU-1, which was stimulated by hydrogen peroxide (H2O2), resveratrol suppressed DNA-synthesis and generation of endogenous O₂, but stimulated NOS activity, which may have been responsible for inhibition of SNU-1 proliferation [14]. Resveratrol also inhibited the growth of esophageal cancer cell line EC-9706 [15].

Resveratrol participates in the prevention of carcinogenesis by inhibition of P450, an enzyme of phase I [16, 17], and through induction of phase II xenobiotic metabolizing enzymes [18]. Resveratrol can induce the activation of p53 and the subsequent apoptosis occurring through p53-dependent pathway [19], but it can also induce apoptosis independently of p53 [20]. Resveratrol can modulate signal transduction pathways by the inhibitory effect on the activation of transcription factors such as NF- κ Band AP1 [18].

Resveratrol, as a selective inhibitor of cyclooxygenase-2 (COX-2), is also a strong inhibitor of the dioxygenase activity of lipoxygenase (LOX) [21]. Atten MJ et al. demonstrated that resveratrol treatment significantly inhibited PKC activity of KATO-III human gastric adenocarcinoma cells and of human recombinant PKC-a [13]. Holian O et al. found that resveratrol stimulated NOS (nitric oxide synthase) activity in human gastric adenocarcinoma SNU-1 cells [14]. They suggested that the antioxidant action of resveratrol toward gastric adenocarcinoma cells might reside in its ability to stimulate NOS to produce low levels of NO (nitric oxide), which, in turn, exerts antioxidant action. Thus, whether resveratrol induces or inhibits NO production depends on the cell system, inducer, and other conditions.

Conclusions

Polyphenolic compounds and antioxidants exert a protective action against gastric cancer.

Resveratrol induces micronuclei in adenocarcinoma cell culture, dose-dependently. An increase number of micronuclei indicates genotoxic activity of resveratrol, the high incidence is at 20 µg/mL resveratrol.

Resveratrol is possible to be used as a cancer chemopreventive or even cancer therapeutic agent. Therefore, agents like resveratrol inhibit proliferation of transformed gastric epithelial cells while remaining relatively nontoxic to the host may constitute a new and effective defense against gastric carcinogenesis.

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