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STUDY ON THE ANTIOXIDANT PROPERTIES OF RESVERATROL IN ONCOLOGY PROPHYLAXIS

STUDIU PRIVIND PROPRIETĂŢILE ANTIOXIDANTE ALE RESVERATROLULUI ÎN PROFILAXIA ONCOLOGICĂ

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ABSTRACT

Cancer is a systemic disease with a huge plurality of clinical manifestations. Chemoprevention is a common approach in this disease, constantly being investigated many substances with prophylactic antitumor properties. Polyphenolic compounds, including resveratrol, have been extensively studied due to their antioxidant action, which is beneficial in chemoprevention. Resveratrol acts as an anticancer agent, suppressing the metabolic activity of procarcinogens by modulating the metabolic enzymes responsible for their activation and by activating antioxidant enzymes. *In vitro* and *in vivo* studies have shown that resveratrol can act as both a chemoprophylactic and a therapeutic agent, being involved in the inhibition of the three developmental stages of neoplasia.

The aim of this study was to evaluate the antioxidant effect of resveratrol, by evaluating three indices of oxidative stress, namely malondialdehyde (MDA), thiol groups (SH), and total antioxidants (AO), in Wistar rats that developed the Walker 256 tumour. The groups benefited from various treatment regimens that included, along with resveratrol, a chemotherapeutic. The results obtained in the study are in favour of the introduction of antioxidants in prophylaxis.

Keywords: antioxidants, cancer, chemoprevention, resveratrol

REZUMAT

Cancerul reprezintă o maladie sistemică, cu o pluralitate imensă de manifestări clinice. Chemoprevenția reprezintă o abordare frecvent întâlnită în această maladie, fiind cercetate numeroase substanțe cu proprietăți antitumorale profilactice. Compușii polifenolici, printre care și resveratrolul, au fost intens studiați datorită acțiunii antioxidante, ce s-a dovedit a fi benefică în chemoprevenție. Resveratrolul acționează ca agent anticancerigen, suprimând activitatea metabolică a procarcinogenilor prin modularea enzimelor metabolice responsabile de activarea lor și prin activarea enzimelor antioxidante. Studiile *in vitro* și *in vivo* au demonstrat ca resveratrolul poate acționa atât ca agent chemoprofilactic cât și terapeutic fiind implicat în inhibarea celor trei faze de dezvoltare ale neoplaziei.

Scopul acestui studiu a fost acela de a evalua efectul antioxidant al resveratrolului, prin evaluarea a trei indici de stres oxidativ, respectiv malondialdehida (MDA), grupările thiol (SH) și antioxidanții totali (AO), la șobolani din rasa Wistar care au dezvoltat tumora Walker 256. Loturile constituite au beneficiat de diferite scheme de tratament care au inclus, alături de resveratrol și un chimioterapic. Rezultatele obținute în cadrul studiului sunt în favoarea introducerii antioxidanților în profilaxie.

Cuvinte cheie: antioxidanți, cancer, chemoprevenție, resveratrol

Cancer, as a biological phenomenon, is one of the main morbid entities, as a degenerative disease, with a serious evolution, which is why it is trying constantly to develop new prophylactic and therapeutic protocols.

Excluding the onset of the disease by the action of determinant oncoinductive agents, there is indisputable evidence that diet plays an important role in both carcinogenesis and chemoprevention (8).

A category of organic compounds intensively studied in recent decades is represented by polyphenols, so far identifying over 8000 substances (2, 18).

Epidemiological studies show that some of them have antioxidant and antiinflammatory properties (30, 33) thus protecting the body against cardiovascular disease, neurodegenerative diseases, type II diabetes, osteoporosis, pancreatitis, bronchopneumonia, and cancer (11, 12, 26, 34).

One of the intensively studied polyphenols, both *in vitro* and *in vivo*, is resveratrol which has been shown to have multiple antitumor effects, both prophylactic and curative (3, 4, 25).

Jang et al. (1997) were the first to show that resveratrol can act as a chemopreventive agent when they found that topical application was able to inhibit tumour formation in the skin cancer model in mice.

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Subsequent studies have shown the involvement of resveratrol in all three evolutionary stages of cancer (20, 21). Initially, resveratrol is involved in inhibiting the activity and expression of certain cytochrome P450 enzymes and increasing the activity of phase II detoxifying enzymes, which convert carcinogens into less toxic and soluble products (13, 17) thus preventing damage to normal cell DNA (6, 9, 14, 19, 27, 28, 32).

Tumour promotion involves clonal enlargement of the initiated cells to create a continuous proliferating, premalignant lesion. Tumour promoters generally alter gene expression, leading to increased cell proliferation and decreased cell death (23). Studies have found that resveratrol exerts anti-proliferative activity by inducing apoptosis (24) and is also involved in inhibiting the processes of several inflammatory enzymes such as COX and lipoxygenases (LOX) (10, 31).

Tumour progression involves invading and metastasizing it by destroying the basement membrane and extracellular matrix. To support rapid growth, the malignant tumour needs to create its own vascular network, through a process called angiogenesis. Many studies have shown that resveratrol suppresses the development of tumour invasion and metastases by inhibiting signalling pathways associated with epithelial-mesenchymal transition (EMT) (29) and is also involved in inhibiting FGF and VEGF growth factors, causing thus blocking angiogenesis (24).

The present study aimed to analyse the chemopreventive efficacy of polyphenolic compounds by evaluating the antioxidant effects of resveratrol in Wistar rats inoculated with Walker 256 tumour.

MATERIALS AND METHODS

The experiment took place within the Animal facility of the Faculty of Veterinary Medicine in Bucharest, and the biochemical investigations were performed by the team of researchers from the Oncological Institute "Prof. Dr. Alexandru Trestioreanu", Bucharest. The experiment was approved by the Faculty Ethics Commission.

Animals and tumour model used

The animals used in the study come from Animal facility of the "Cantacuzino" Institute, Bucharest. For the study, 30 Wistar outbred rats, males, with bodyweight between 80 - 120 g were used. The deployment conditions were compliant, the animals benefiting from optimal housing and feeding conditions. The accommodation of the animals was made in cages made of resistant material, easy to clean and disinfect, with dimensions of 47x27 cm, 2 per cage. Rats had *ad libitum* access to food and water. During the experiment, their health was monitored daily, and their handling and necessary procedures were performed so as not to cause stress or pain. The tumour model used, represented by Walker 256 carcinoma, came from the experimental tumour biobank of the Oncological Institute "Prof. Dr. Alexandru Trestioreanu" from Bucharest. This is a standard tumor model used in the preclinical screening of antitumor substances as well as in various experimental models of chemo-radiotherapy as well as in lymph node metastasis models (7). The study animals were inoculated subcutaneously in the left flank with a tumour suspension containing 5×10^6 ascites cells of Walker 256 carcinoma, suspended in a volume of 0.5 ml of liquid.

Used substances and therapeutic protocol

Epirubicin is a chemotherapeutic agent from the anthracycline class used in the treatment of several types of cancer. The mechanism of antitumor action is not fully elucidated, but it is thought that, like other anthracyclines, it acts on DNA, inhibiting replication and transcription. The action of epirubicin can be attributed (at least in part) to interference with topoisomerase II (8). Reducing anthracyclines to free radicals can cause damage to DNA, cell membrane lipids, and mitochondria, which is why we created the association between a polyphenol with antioxidant effect, respectively resveratrol and a chemotherapeutic that produces, by metabolism, oxygen-free radicals.

The study was performed on 30 animals that were divided into three equal groups: Group T (control) included animals, with a Walker 256 subcutaneous tumour, who benefited from a standard diet. The TC group (experimental, with cytostatic) consisted of animals who, after the macroscopic appearance of the tumour, were given Epirubicin, at a dose of 5 mg/kg, intratumorally, in 3 courses, at intervals of 7 days. Their diet was standard. The TCR group (experimental, with cytostatic and resveratrol) consisted of animals fed, prophylactically, with resveratrol (20 mg/kg, PO) daily, over 7 days pre-inoculation and 14 days after tumour inoculation, until macroscopic detection of the tumour. Intra-tumoral chemotherapy with Epirubicin (5 mg/kg) was performed in 3 courses every 7 days.

Evaluation of oxidative stress

For the evaluation of oxidative stress, were determined: malondialdehyde (MDA), as a marker of lipid peroxidation, thiol groups, and total antioxidants, these being determined from the serum of the animals studied. MDA was determined by the Carbonneau method, the determination of albumin thiols was done by the Albini method and the determination of total antioxidants was performed by monitoring the ability of the biological sample to reduce iron (FRAS).

Statistical analysis

For the statistical analysis, the Excel program was used, the data were presented as mean ± SD (stan-

dard deviation) and SE (standard error).

RESULTS AND DISCUSSION

Oxidative stress has been reported in almost all cancers, promoting many aspects of tumour development and multiplication. The role of reactive oxygen species in cells is dual, in low concentration being involved in cell signalling, converting cell phenotype to tumour progression by angiogenesis, and in high concentration attests to the destructive effect on tumour cells. The generation of oxidative stress during the process of tumour growth and development is highlighted especially by the stimulation of lipid peroxidation, a process that generates numerous electrophilic aldehydes, stable compounds that can diffuse inside the cell exerting several cytotoxic effects (15, 16, 22). The effect of administering a compound with antioxidant potentials, such as resveratrol, administered at a dose of 20 mg/kg, daily, one week before inoculation of the tumour line in Wistar rats, allowed us to evaluate its involvement in tumour evolutionary stages by assessing the degree of tumour progression given by the values of the tumour volume as well as by the results obtained when evaluating the biochemical parameters of oxidative stress. During the experiment, the tumour volume (Vt) was determined weekly using the formula $D \times d^2 / 2$ (D-large diameter of the tumour, d-small diameter of the tumour), the first measurement is performed at macroscopic detection of the tumour. The graphical evolution of the average Vt, in dynamics, shows a significant difference between the three groups,respectively 10.96 cm^2 in the TCR group, 19.62 cm^2 in the TC group, and 34.59 cm^2 in the T group, which proves the direct involvement of resveratrol in the promotion and progression stages of the tumour by inhibiting tumour development (Fig. 1).

From the point of view of oxidative stress, the three evaluated parameters, respectively malondialdehyde (MDA), thiols (TH), and total antioxidants (AO) supported and completed the results obtained when evaluating the tumour volume. Fig. 2, 3, 4 show the results of the evaluation of MDA, TH, and AO according to each group.

Thus, as shown in Table 1, the lowest values of malondialdehyde (MDA) were recorded in the TC group (7.82 ± 1.07 µmol / 100 ml serum) followed by those of the TCR group (8.13± 0.89 µmol / 100 ml serum), group T having the highest concentration (10.66 ± 1.07 µmol / 100 ml serum). The values obtained, associated with the average of the tumour volumes, attest to the fact that the tumour progression is associated with increased levels of lipid peroxidation. The concentration of thiols in serum (SH) is also correlated with the presence and intensity of oxidative stress, their values being the highest in the TC group where the cytostatic is a producer of reactive oxygen species.

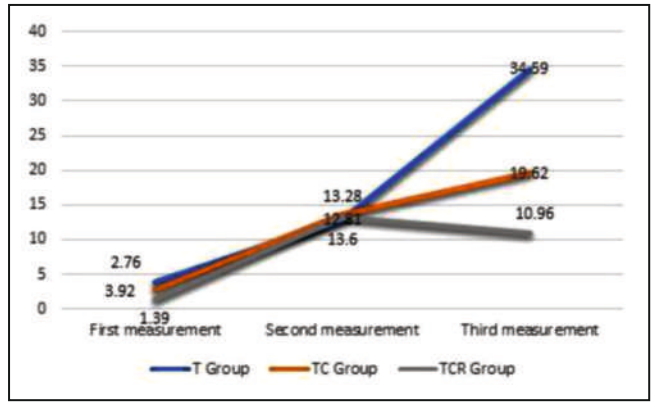

Fig. 1. Graphical representation for the evolution of the average tumour volume, depending on the group

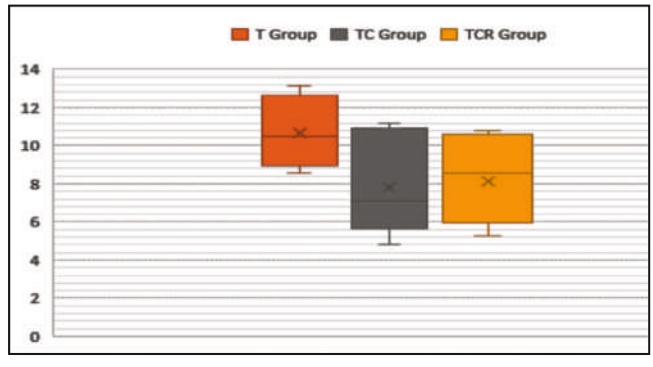

Fig. 2. Graphical representation of the MDA level, depending on the group

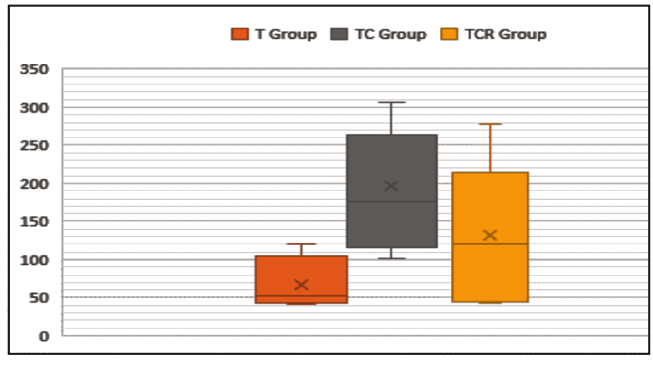

Fig. 3. Graphical representation of albumin thiol values, depending on the group

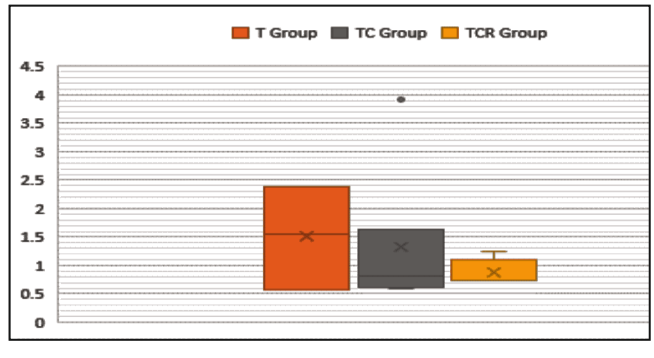

Fig. 4. Graphical representation of total antioxidant values, depending on the group

Table 1

Relationship between tumour volume and oxidative stress assessment

Groups	Tumour volume (cm^3)			MDA (µmol/100 ml ser)	SH (µmol/L)	AO (moli/ml)
	I measurement	II measurement	III measurement			
T Group	3.92±1.6	12.81±4.7	34.59±28.42	10.66±1.07	66.25±18.47	1.5±0.52
TC Group	2.76 ± 1.15	13.60 ± 4.73	19.62±5.92	7.82±1.07	196.85±29.75	1.32±0.45
TCR Group	1.39±0.61	13.28±8.07	10.96±4.57	8.13±0.89	131.14±35.16	0.87±0.09

* Values are presented as mean ± SE (standard error)

In the TCR group, the mean SH values are lower, thus demonstrating the direct involvement of the antioxidant activity of resveratrol. It is also observed that the values of total antioxidants are significantly lower in the TCR group compared to the other two groups thus emphasizing that resveratrol administered preventively has a role in reducing the imbalance created by free radicals released by the tumour process and endogenous antioxidants.

The obtained results by us come to complete the numerous studies on animal models which have shown that the supplementation of the diet with resveratrol decreases the incidence of tumour formation. Thus, Banerjee et al., in 2002, showed that dietary supplementation with resveratrol in 45-day-old Sprague -Dawley female rats, started 7 days before tumour onset and continued for 120 days after initiation, increased the time to the first formation of the breast tumour and decreased the tumour multiplication (1).

Using five-week-old Sprague-Dawley rats and a DMBA carcinogenesis model, Chatterjee et al. (2011) found that dietary supplementation with resveratrol decreased the incidence of palpable breast tumour (5).

In another study, performed on female nude athymic mice, Garvin et al. found that resveratrol (25 mg/kg) given daily for 3 weeks after the tumours had already reached 40 mm3 caused a significant reduction in tumour growth. Moreover, apoptosis increased and angiogenesis decreased in resveratrol tumour cells compared to mice not administered (14). All these studies, together with the results obtained by us, suggest that resveratrol can be used as a chemopreventive agent or can be used as an adjunct in cancer therapy.

CONCLUSIONS

The analysis of oxidative stress is essential not only to assess the state of the disease but also to develop new preventive and therapeutic strategies, based on the action of antioxidants.

In our study of Wistar rats inoculated with Walker 256 tumour, we evaluated the efficacy of resveratrol in chemoprevention. The results obtained after prophylactic administration of resveratrol in animals that develop a neoplastic disease, clearly show that the introduction into the diet of compounds with antioxidant potential is essential in regressing tumor progression by reducing the imbalance caused by prooxidants generated by the tumour.

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