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Research Article

Study on the Use of Resveratrol as an Adjuvant in Cancer Therapy

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Abstract

Chemotherapy, the most widely used therapy in oncology, leads to the accumulation of reactive oxygen species, which often generates adverse effects in patients. To reduce the oxidative stress due to either the action of cytostatics or pathological tumour mechanisms, we analyzed the effectiveness of antioxidants. The use of antioxidants as adjuvant therapy can improve the therapeutic effects by potentiating the antitumour action of cytostatics and reducing their toxicity to healthy tissues.

The aim of this study was to evaluate the efficacy of resveratrol administered 3 days after Epirubicin in Wistar rats inoculated with Walker tumour 256. In our experiment we evaluated three indices of oxidative stress and found that the lowest values were recorded at the group receiving resveratrol, thus emphasizing the beneficial antioxidant action of resveratrol.

Keywords: Antioxidants; Cancer; Epirubicina; Resveratrol; Walker 256 Tumour

Abbreviations

ROS: Reactive Oxygen Species; MDA: Malondialdehyde; SH: Thiol Groups; AO: Total Antioxidants

Introduction

Cancer is an extremely complex pathology that requires a multimodal therapeutic approach that is often individualized depending on the situation (tumour type, stage, patient, etc.). Although cytostatics are associated with a multitude of side effects because they attack both tumour cells and healthy cells, chemotherapy is the most widely used therapy against cancer. Each substance can cause various side effects that lead to deterioration in the patient's quality of life [11,16]. One of the major causes of side effects, especially for DNA-targeted substances, is the overproduction of reactive oxygen species (ROS) and the onset of oxidative stress [16]. Often, chemotherapy-mediated toxicities are related to the generation of ROS, chemotherapy becoming an indirect but substantial source of free radical generation [10].

Some of the drugs used in chemotherapy that cause high levels of oxidative stress are based, in part, on the use of this stress mechanism to destroy tumour cells [3,13]. In order to reduce the side effects of chemotherapy, several substances have been tested,

of which antioxidants have been shown to be beneficial, being increasingly recommended as adjuvants in chemotherapy. Antioxidants prevent cell damage by eliminating free radicals, but the interaction between chemotherapy and antioxidants is more complex than simply promoting and inhibiting oxidative stress. Each antioxidant has a different interaction depending on the dose and the cytostatics with which it interacts [1,5].

The use of antioxidants concomitantly with chemotherapy has been avoided due to the mechanism of action of certain antineoplastic agents, which involves the generation of free radicals. Antioxidants can interfere with some cytostatics, decreasing their effectiveness [16]. However, various substances with an antioxidant role are increasingly being used as adjuvant therapy, thus allowing patients to tolerate higher doses of cytostatics, increasing the chance of a better tumour response and improving the patient's survival time. Specialist studies have shown that high levels of antioxidants can protect against oxidative stress induced by chemotherapy in patients diagnosed with cancer [5].

Antioxidants can be used in cancer therapy either as chemoprevention (using preventive, low doses) or with therapeutic role (using a much higher therapeutic dose). Numerous studies have analysed if antioxidants can protect healthy tissue without influencing the effectiveness of cytostatics. And the latest research has shown that they can protect healthy tissue, increasing the effect of chemotherapy and allowing the use of higher doses [14,15].

Aim of the Study

This study aimed to analyze the antioxidant effects of resveratrol in advanced stages of carcinogenesis in Wistar rats inoculated with Walker 256 tumour. In this experiment we evaluated three indices of oxidative stress, namely malondialdehyde (MDA), thiol groups (SH) and total antioxidants (AO), analyzing the effect of resveratrol on the chemotherapy-induced toxicities.

Materials and Methods

The study was performed within the Biobase of the Faculty of Veterinary Medicine Bucharest, and the biochemical investigations were performed within the Oncological Institute "Prof. Dr. Alexandru Trestioreanu". This experiment was approved by the Ethics Commission of the Faculty of Veterinary Medicine.

Tumour model and animals used

The experiment used 30 Wistar rats, young, male, with body weight between 80 and 120g. They came from the biobase of the Cantacuzino Institute in Bucharest. The conditions of the experiment were compliant, the animals benefited from optimal accommodation and feeding conditions. The animals were housed 2 per cage and they had ad libitum access to food and water. During the study, the animals' health was daily monitored, and no pain or stress was inflicted during the necessary procedures and handling of animals.

For this study, we used the Walker 256 carcinoma, the tumour model came from the experimental tumour biobank of the Institute of Oncology "Prof. Dr. Alexandru Trestioreanu" from Bucharest. This tumour model is frequently used in antitumour susbstances research, in various experimental models of chemotherapy (Comişel V., 1999). The animals were inoculated subcutaneously in the left flank with a tumour suspension containing 5 x 10^6 ascites Walker 256 carcinoma cells, suspended in 0.5 ml of liquid.

Therapeutic protocol and used substances

In this experiment, we used Epirubicin, which belongs to the class of anthracyclines and is a frequently administered cytostatic in the therapy of neoplasms. The mechanism of action of epirubicin is complex, it inhibits replication and transcription, interferes with topoisomerase II and produces oxygen free radicals. The antitumour action is manifested in the DNA, but free radicals can cause alterations in the mitochondria or lipids of the cell membrane [6].

As an antioxidant we chose the use of a polyphenolic compound, respectively resveratrol, this being a highly researched natural compound, which has proven its effectiveness both in the prevention of many pathologies, including cancer, and in their therapy (being included in various therapeutic protocols). Numerous studies have shown that resveratrol has multiple anticancer effects, protecting against both tumour initiation and multiplication [4].

This study was performed on a number of 30 animals, divided into three groups of 10 animals each. Group C (control) comprised animals with Walker 256 subcutaneous tumour and no treatment, representing the control group. The TC group consisted of animals who were given intratumourally Epirubicin after the macroscopic appearance of the tumour (at a dose of 5 mg/kg, 3 administrations every 7 days). The RE group (resveratrol and epirubicin) consisted

of animals who were given Resveratrol (20 mg/kg, PO) 3 days after Epirubicin administration.

Evaluation of oxidative stress

Evaluation of oxidative stress was performed by analyzing total antioxidants, thiol groups and malondialdehyde (MDA). The determination of the parameters was made from the serum collected from the animals. The Carbonneau method was used to determine malondialdehyde, the Albini method was used for albumin thiols 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 we used the Excel program, the data were presented as mean ± SD (standard deviation). The differences between the mean values were analyzed using the unidirectional Anova test, the p value ≤ 0.05 being considered statistically significant.

Results and Discussion

Oxidative stress is involved in all aspects of tumour development and progression and often in anti-cancer therapies. During the process of carcinogenesis, an increase in the levels of reactive oxygen species (ROS) may occur due to increased metabolic activity, oncogene activation, increased cellular receptor signal or mitochondrial dysfunction. ROS overproduction can also be exogenously induced by various drug substances or other external factors [2]. Epirubicin is one of the drugs that contributes to the increase of ROS, acting as a cytostatic agent through two mechanisms (inhibition of DNA replication and transcription and accumulation of reactive oxygen species).

Direct measurement of the levels of reactive oxygen species is difficult due to their reactivity and short lifespan, which is why the analysis of oxidative damage caused by these radicals to lipids, proteins and cellular nucleic acids is used. Lipid peroxidation is frequently used as an indicator of ROS-mediated damage to cell membranes and the most studied end product of polyunsaturated fatty acid peroxidation is malondialdehyde [9].

By evaluating the serum lipid peroxidation levels in Wistar rats, a significant decrease in MDA was observed in the RE group. Figure 1 shows the evolution over time (three measurements were made at a difference of 7 days) of MDA serum concentration, depending on the group, noting that both TC group and RE group showed a constant decrease in values of MDA. It is known that lipid peroxides increase in the first phases of tumour growth, subsequently registering a decrease (without reaching normal values) [8], this aspect being observed from the evolution of MDA recorded in group C. Following the unidirectional Anova test, the p value recorded was 0.009 ($p < 0.05$), indicating that the differences recorded between batches are statistically significant.

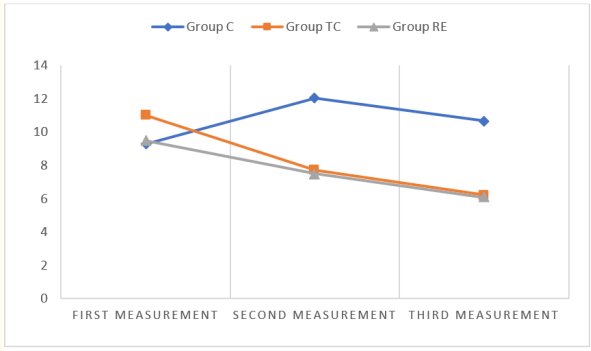

Figure 1: Graphical representation for the evolution of MDA serum concentration, depending on the group.

The results obtained from the analysis of total antioxidants, thiol groups and malondialdehyde demonstrate the influence of resveratrol on oxidative stress, noting that its administration led to a significant decrease in the analyzed parameters (Table 1). The effectiveness of the administration of antioxidants as adjuvants can be evaluated through analyzing oxidative stress. In this study we observed that the lowest values of the parameters analyzed were at the group in which we administered resveratrol after treatment with Epirubicin.

	MDA (µmol/100 ml)	SH (µmol/L)	AO (moli/ml)
Group C	10.66 ± 1.62	66.25 ± 36.94	1.5 ± 0.91
Group TC	8.32 ± 2.2	196.85 ± 78.72	1.32 ± 1.19
Group RE	7.526 ± 1.54	191 ± 126.27	1.032 ± 0.56

Table 1: Evaluation of oxidative stress, depending on the group. *values are presented as mean ± SD (standard deviation).

Figure 2-4 present graphically the results obtained from the analyzes performed to evaluate the oxidative stress, in batches. As shown in the graphs and table, malondialdehyde and total antioxidants show the lowest values in the RE group, indicating that resveratrol helps reduce the imbalance created by free radicals formed by the tumour process and the use of cytostatic.

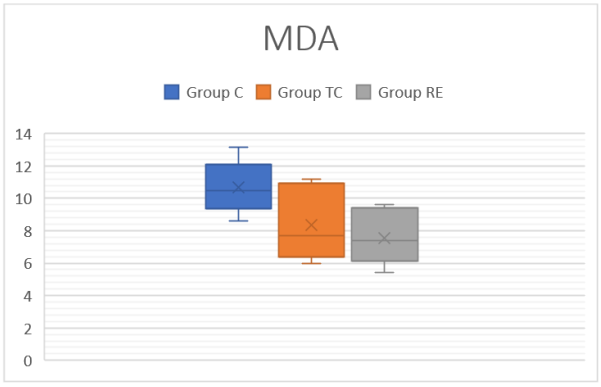

Figure 2: Graphical representation of the MDA level, depending on the group.

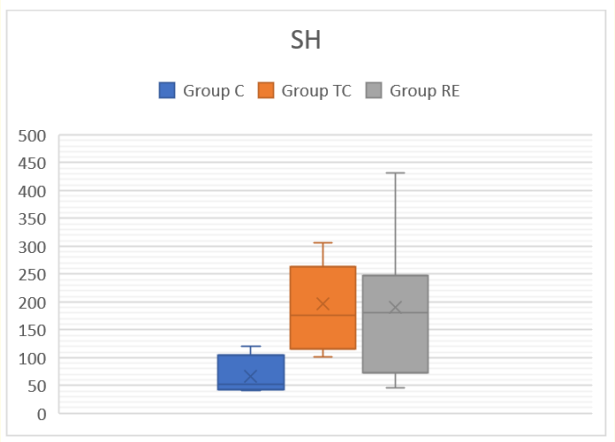

Figure 3: Graphical representation of the albumin thiol values, depending on the group.

All 3 groups consisted of 10 Wistar rats with Walker 256 tumour (macroscopically detectable). During the experiment, we noticed that in the RE group no rat showed exitus, unlike the other groups,

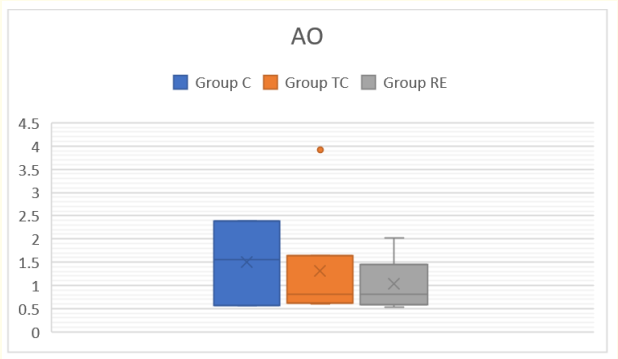

Figure 4: Graphical representation of total antioxidants values, depending on the group.

where from group C four died and from group TC two rats died. From this observation it is highlighted that the administration of antioxidants has a beneficial intake, also contributing to increasing the survival time of patients.

The results obtained in this study come to complete the numerous studies performed to highlight the effectiveness of antioxidants in cancer therapy. Nechuta., *et al*. (2011) observed that the use of supplements (vitamin E, vitamin C, antioxidants) in the first 6 months after the diagnosis of breast cancer may be associated with a reduced risk of mortality and recurrence. Chemotherapy reduces serum levels of antioxidant vitamins and minerals and helps to achieve a higher level of oxidative stress. Therefore, the supplementation of certain antioxidants and nutrients can contribute to improving the health of patients undergoing continuous chemotherapy [7,12]. Singh., *et al*. evaluated the efficacy of antioxidants in supplementing chemotherapy by analyzing 174 original articles. By comparing the results, they observed that 88% of the articles are studies that state that antioxidants attenuate from the toxicity induced by cytostatic agents and 70% of research have reported that the therapeutic efficacy of chemotherapy increases in the presence of antioxidants. Also, 63% of the articles stated that the use of antioxidants in cancer therapy increases the survival time of patients. Therefore, Singh., *et al*. concluded that adjuvant therapies that use antioxidants or their precursors do not interfere with chemotherapy and that they are beneficial in the therapy of neoplasms, thus improving therapeutic efficacy [16].

Conclusion

Evaluation of oxidative stress in oncology contributes to the development and improvement of therapeutic strategies. Various antioxidant substances can be successfully used to reduce the accumulated oxidative stress either due to the oncological condition or due to the administered cytostatics.

In our study with Wistar rats inoculated with Walker 256 tumour, we observed that the introduction of resveratrol into the therapeutic protocol had favorable results. The efficacy and antioxidant action of resveratrol is evident from the results of the evaluation of oxidative stress. Knowing that the excess production of reactive oxygen species and the installation of oxidative stress contribute to the initiation and progression of the tumour and observing from the experiment performed that the group that received antioxidants showed the lowest values for the analyzed parameters we can say that the use of antioxidants as adjuvant therapy has a beneficial contribution in oncological therapeutic management.

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Conflict of Interest

There is no financial interest or any conflict of interest.

Bibliography

1. Alexieva B., *et al*. "Free radicals, antioxidants and cancer chemotherapy". *Acta Medica Kinki University* 35 (2010): 57-65.
2. Andrisic L., *et al*. "Short overview on metabolomics approach to study pathophysiology of oxidative stress in cancer". *Redox Biology* 14 (2018): 47-58.
3. Block KI., *et al*. "Impact of antioxidant supplementation on chemotherapeutic toxicity: a systematic review of the evidence from randomized controlled trials". *International Journal of Cancer* 123 (2008): 1227-1239.
4. Carter LG., *et al*. "Resveratrol and cancer: focus on *in vivo* evidence". *Endocrine-Related Cancer* 21.3 (2014): R209-R225.
5. Conklin KA. "Chemotherapy associated oxidative stress: impact on chemotherapeutic effectiveness". *Integrative Cancer Therapies* 3 (2004): 294-300.
6. Crînganu D. "Oncologie generală - Patologia animalelor de companie". Ed. Printech, Bucureşti 1 (2009).
7. Drisko JA., *et al*. "The use of antioxidant therapies during chemotherapy". *Gynecology and Oncology* 88 (2003): 434-439.
8. Gruia Maria Iuliana., *et al*. "Evaluation of antioxidants role in diminution of lipid peroxidation at tumour bearer mice". *Romanian Journal of Comparative Oncology* 4 (2001): 268-273.
9. Katerji M., *et al*. "Approaches and methods to measure oxidative stress in clinical samples: Research applications in the cancer field". *Oxidative Medicine and Cellular Longevity* (2019).
10. Kunwar A and Priyadarsini KI. "Free radicals, oxidative stress and importance of antioxidants in human health". *Journal of Medical and Allied Sciences* 1 (2011): 53-60.
11. Lamson DW and Brignall MS. "Antioxidant in cancer therapy: their actions and interactions with oncologic therapies". *Alternative Medicine Review: a Journal of Clinical Therapeutic* 4 (1999): 304-329.
12. Nechuta S., *et al*. "Vitamin supplement use during breast cancer treatment and survival: a prospective cohort study". *Cancer Epidemiology, Biomarkers and Prevention* 20 (2011): 262.
13. Shinde A., *et al*. "Effect of free radicals and antioxidant on oxidative stress: a review". *Journal of Dental and Allied Sciences* 1 (2012): 63-66.
14. Simone CB., *et al*. "Antioxidants and other nutrients do not interfere with chemotherapy or radiation therapy and can increase kill and increase survival, part 1". *Alternative Therapies in Health and Medicine* 13 (2007a): 22-28.
15. Simone CB., *et al*. "Antioxidants and other nutrients do not interfere with chemotherapy or radiation therapy and can increase kill and increase survival, part II". *Alternative Therapies in Health and Medicine* 13 (2007b): 40-47.

16. Singh K., *et al*. "Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity-Exploring the armoury of obscurity". *Saudi Pharmaceutical Journal* 26.2 (2018): 177-190.

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