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**THE EFFECT OF BRACHYTHERAPY ON ANTIOXIDANT STATUS
AND LIPID PEROXIDATION IN PATIENTS WITH CANCER OF THE
UTERINE CERVIX**

CELESTYNA MILA-KIERZENKOWSKA^{1*}, KORNELIA KEDZIORA-
KORNATOWSKA², ALINA WOŹNIAK¹, TOMASZ DREWA^{1,3}, BARTOSZ
WOŹNIAK⁴, SYLWIA DREWA⁵, EWA KRZYŹYŃSKA-MALINOWSKA¹
and ROMAN MAKAREWICZ⁶

¹Department of Medical Biology, ²Clinic of Geriatrics, ³Clinic of Urology,
⁴Clinic of Neurosurgery and Neurotraumatology, ⁵Department of Radiology,
Ludwik Rydygier Medical University, Karłowicza 24, PL-85-092 Bydgoszcz,
Poland, ⁶Regional Centre of Oncology, Romanowskiej 2, PL-85-796 Bydgoszcz,
Poland

Abstract: The aim of this study was to investigate the effect of brachytherapy on lipid peroxidation and antioxidant status in patients with uterine cervix cancer. The study was conducted on 84 uterine cervix cancer patients from the Brachytherapy Department of the Regional Centre of Oncology in Bydgoszcz. Patients with uterine cervix cancer were found to have elevated levels of lipid peroxidation and antioxidant defence system impairment relative to healthy females. The results of the study indicate that brachytherapy has no direct effect on the antioxidant system of patients with uterine cervical carcinoma. However, the normalisation of catalase and glutathione peroxidase activity and erythrocyte TBARS level observed six months after the end of therapy may be due to the arrest of the progression of the disease.

Key Words: Free Radicals, Catalase, Glutathione Peroxidase, Lipid Peroxidation, Cervical Cancer, Brachytherapy

* Corresponding author; tel: 48 52 585 3737, fax: 48 52 585 3742, e-mail: celestyna@go2.pl

Abbreviations used: CAT – catalase; GSH-Px – glutathione peroxidase; ROS – reactive oxygen species; MDA – malondialdehyde; TBARS – thiobarbituric acid reactive substances; LDR – low dose rate; NADPH – reduced nicotinamide adenine dinucleotide phosphate.

INTRODUCTION

Cancer of the uterine cervix is the leading malignancy affecting women in the world today. Although a number of risk factors have been identified, the aetiology of the disease is not yet clearly understood.

Radiation therapy is one of the most standard and effective modalities for the current treatment of cervical cancer. It has proven to be as effective as surgery for the treatment of early stage cervical cancer [1], and it remains the most commonly used modality for the treatment of inoperable cervical cancers [2]. A better understanding of the response of tumors to ionizing radiation might potentially lead to an improvement in tumor control and patient morbidity [3].

Radiation-induced oxidative damage to cells has been receiving growing attention from molecular radiobiology researchers. The physiological and cellular changes such as increased synthesis of specific cellular antigens and damage to cellular DNA have been well documented for irradiated organisms [4, 5]. The damaging effects of irradiation are brought about through both direct and indirect mechanisms [6]. The direct effects and the overall energy deposit process along the radiation track lead to complex and multiple reactions involving ionization, secondary electrons and excitation processes. The indirect effects are thought to be caused by the production of highly reactive free radicals, which can subsequently damage critical biological molecules. One of the most important radiation-induced reactive oxygen species (ROS) is the hydroxyl radical, which is generated by ionizing radiation either directly by the oxidation of water, or indirectly by the formation of secondary partial ROS [7]. Organisms have developed a comprehensive array of antioxidant defences to prevent free radical formation or limit their damaging effects. These include enzymes such as superoxide dismutase (SOD) and catalase (CAT) or glutathione peroxidase (GSH-Px) as means of eliminating the superoxide radical ($O_2^{\cdot-}$) or hydrogen peroxide (H_2O_2), respectively [8]. In the presence of H_2O_2 and the ions of transitional metals, $O_2^{\cdot-}$ is converted to the highly reactive hydroxyl radical (Fenton's and Haber-Weiss's reactions) [8], thus elimination of $O_2^{\cdot-}$ and H_2O_2 by antioxidant enzymes prevents hydroxyl radical formation. In recent years, there has been a growing interest in studying the role played by lipid peroxidation products and antioxidants in cancer patients. An impairment of the antioxidant defence system has been implicated in many diseases including cancers, and the activities of enzymic antioxidants have shown different patterns during neoplastic transformation. Antioxidant enzyme activities are highly variable in tumor tissue and in the blood of patients with different types of cancer [9-15].

The aim of this study was to evaluate the effects of brachytherapy on the activity of antioxidant enzymes and the levels of lipid peroxidation products in uterine cervix cancer patients.

MATERIALS AND METHODS

This study was conducted on patients with carcinoma of the uterine cervix attending the Brachytherapy Department of the Regional Centre of Oncology in

Bydgoszcz. Blood samples were obtained from 84 uterine cervix cancer patients with an average age of 51 (ranging from 28 to 78). As per the treatment protocol followed at the Brachytherapy Department of the Regional Centre of Oncology in Bydgoszcz, all the patients were treated by intracavitary irradiation using a Selectron LDR brachytherapy unit. The patients underwent brachytherapy twice with an interval of 7 to 10 days. The dose given was from 1500 cGy for reference point A to 3000 cGy for reference point A.

The blood samples were collected before giving radiotherapy, the day after each brachytherapy treatment and about six months after the end of brachytherapy. One set of control blood samples were obtained from 30 healthy females without any known disease with an average age of 43 (ranging from 20 to 71). Catalase and glutathione peroxidase activities were measured in the erythrocytes, while the thiobarbituric acid reactive substances level was analyzed both in the erythrocytes and in the blood plasma.

The Beers and Sizer method [16] was used to determine the catalase activity. This method is based on the measurement of the absorbance decrease of hydrogen peroxide which is decomposed by catalase, measured at a wavelength of 240 nm. The CAT activity was expressed as 10^4 IU/g Hb.

Glutathione peroxidase activity was assayed according to Paglia and Valentine [17]. This method is based on the measurement of changes in absorbance at a wavelength of 340 nm, caused by the oxidation of reduced nicotinamide adenine dinucleotide phosphate. NADPH is a coenzyme of reduction of glutathione disulphide. The obtained oxidized glutathione is a product of the reaction catalysed by glutathione peroxidase. The activity of GSH-Px was expressed as U/gHb.

Thiobarbituric acid reactive substances level was determined according to the method of Buege and Aust [18]. This method involves the creation of a coloured complex between lipid peroxidation products and thiobarbituric acid at a temperature of 100°C and in an acidic environment. The maximum absorption of that complex at a wavelength of 532 nm was recorded. The main thiobarbituric acid reactive substances are malondialdehydes, so the TBARS concentration in the plasma was expressed as nmol of MDA/ml and in the erythrocytes as nmol of MDA/g Hb.

The values obtained were statistically analyzed using the one-way ANOVA test. A result of $p < 0.05$ was considered significant.

RESULTS

The activity of catalase in the control group amounted to 50.63×10^4 IU/g Hb, which was lower than the CAT activity observed for the erythrocytes of cervical cancer patients both before and in the course of treatment (Tab. 1). Catalase activity was found to be unaltered during brachytherapy, while six months after the end of the therapy, CAT activity was found to have reduced to 58.42×10^4 IU/g Hb: about 14-18 % lower than for patients during treatment. These differences were found to be statistically significant ($p < 0.001$).

Tab. 1. The activity of catalase and glutathione peroxidase in erythrocytes and the concentration of thiobarbituric acid reactive substances both in the blood plasma and in the erythrocytes of women with cervical cancer treated via brachytherapy; compared to results for the control group.

	CAT activity [x 10 ⁴ IU/g of Hb] ± SD	GSH-Px activity [U/g Hb] ± SD	plasma TBARS level [nmol MDA/ml] ± SD	erythrocyte TBARS level [nmol MDA/g Hb] ± SD
controls	50.63 ± 11.50	15.15 ± 4.86	0.42 ± 0.19	32.42 ± 8.24
before therapy	68.09 ± 17.05 ◆◆◆	10.07 ± 3.06 ◆	0.54 ± 0.16 ◆◆	39.83 ± 15.91 ◆
1 day after 1st treatment	68.28 ± 18.29 ◆◆◆	10.87 ± 5.12 ◆	0.55 ± 0.15 ◆◆◆	44.94 ± 20.15 ◆◆
before 2nd treatment	70.72 ± 22.11 ◆◆◆	10.44 ± 4.92 ◆	0.56 ± 0.16 ◆◆◆	45.85 ± 23.94 ◆
1 day after 2nd treatment	71.39 ± 19.07 ◆◆◆	9.72 ± 2.89 ◆◆	0.56 ± 0.15 ◆◆◆	43.52 ± 20.03 ◆
six months after therapy	58.42 ± 19.62	13.99 ± 3.15	0.53 ± 0.11 ◆◆◆	35.73 ± 13.59

◆ - compared to control group p<0.05; ◆◆ - p<0.01; ◆◆◆ - p<0.001

In the control subjects, glutathione peroxidase activity amounted to 15.15 U/g Hb and was statistically significantly higher than in patients with cervical cancer before and in the course of treatment (Tab. 1). However, no remarkable alterations in the activity of glutathione peroxidase were found at different points of the treatment. The activity of glutathione peroxidase was found to be increased significantly to 13.99 U/g Hb in patients who had completed the treatment and was about 30-40% higher than in patients before and during brachytherapy. These differences were found to be statistically significant (p<0.05).

The control subjects were found to have a lower plasma TBARS level than the patients with cancer of the uterine cervix at all the analyzed points of treatment (Tab. 1). The level of TBARS was not altered in subjects from the cervical cancer patients group.

The level of TBARS in the erythrocytes of cervical cancer patients was statistically significantly higher than that of the control subjects, while no statistically significant differences in the erythrocyte TBARS level were observed for patients during brachytherapy (Tab. 1). The lowest erythrocyte

TBARS level was revealed six months after the completion of brachytherapy and it was 35.73 nmol/gHb.

DISCUSSION

This study shows how antioxidant defence mechanisms are impaired in human uterine cervical carcinoma. Compared to the control group, the activity of glutathione peroxidase was found to be remarkably reduced while the activity of catalase was significantly elevated in patients with cervical cancer. Although both GSH-Px and CAT catalyze H₂O₂ breakdown, GSH-Px has a higher affinity for the substrate at physiological concentrations of H₂O₂ generation and can scavenge a wide range of hydroxyperoxides [8].

Extensive work has been carried out on the relationship between free radical activity, antioxidants scavenging of free radicals, and cancer of the uterine cervix, but the presented results are not unequivocal. A significantly increased level of lipid peroxidation with a concomitant decrease in antioxidant levels in cervical cancer patients relative to normal subjects and patients with cervicitis was observed by Manoharan *et al.* [19]. Impaired antioxidant status in the carcinoma of the cervix was also demonstrated by Ahmed *et al.* [20]. The decrease in antioxidant enzyme activity may be attributed reduced synthesis of said enzymes in the tumor tissue. The elevated lipid peroxidation level in turn may be due to extensive tissue damage or a decrease in the efficacy of the antioxidant defence mechanism.

Relative to the control subjects, significantly elevated levels of plasma thiobarbituric acid reactive substances (TBARS) and impairment of antioxidant defence mechanisms were found for cervical cancer patients [21]. Disturbed antioxidant enzyme activity and elevated lipid peroxidation were also reported for patients with uterine cervicitis and myoma, which often lead to cervical cancer [22].

In our study, significantly higher levels of lipid peroxidation products were observed both in the plasma and erythrocytes of cervical cancer patients than in those of the control group. A reason for this increased lipid peroxidation in the circulation of cervical cancer patients may be a poor enzymatic and non-enzymatic antioxidant defence system [23]. This may also be due to excessive generation of lipid peroxidation products in tumor tissues, and the subsequent release of these products into the circulation. An increased lipid peroxide level in proliferating cells leads to an increase in the serum lipid peroxide level in cancer patients [24].

In our study, the effect of gamma radiation on the status of antioxidant enzymes and the lipid peroxidation process in cervical cancer patients was investigated. It is known that radiation produces effects on the cell membrane and cytoplasmic organelles, causing various alterations, such as signalling, gene expression and cell cycle regulation. The major radiation effects are believed to be mediated by the indirect actions of those free radicals, which diffuse and react with vital

biological molecules [25], as well as by the hydroxyl radical, which indiscriminately attacks neighbouring molecules often at near diffusion-controlled rates [7]. Radiation-induced oxidative damage has shown that peroxidative processes produce membrane structural changes and cause cellular injury [25].

The data from this investigation did not reveal any significant relationship between the treatment response and the changes in antioxidant enzyme activity and the levels of lipid peroxidation products in the blood of cervical cancer patients. There were no significant changes in the activity of CAT and GSH-Px or in the level of TBARS on the first and on the seventh day after the first brachytherapy treatment nor after the second brachytherapy treatment. Neither were remarkable alterations in the levels of plasma GSH, erythrocyte GSH or GPX activity reported by Mukundan *et al.* [26] at two different time points after treatment; this observation partially concurs with our findings. In our study, no changes in antioxidant enzyme activity were observed during brachytherapy, but the activity of CAT and GSH-Px in patients after the treatment returned to normal.

The presented results may indicate the lack of a visible effect on oxidant and antioxidant activity changes in patients with cervical carcinoma treated with brachytherapy. It is possible that in our study, an enormous production of free radicals after gamma radiation exposure occurs only in the tumor tissue, and not in the system. The period between the first and the eighth day after applying the first brachytherapy treatment is too short for changes that have occurred in the tumor tissue as a result of the radiation to be reflected in the activity of antioxidant enzymes or in the levels of lipid peroxidation products in the blood of patients.

The effect of radiotherapy on the circulating antioxidant system of human uterine cervical carcinoma was also demonstrated by Bhuvaramurthy *et al.* [27]. The activities of CAT, SOD and GSH-Px and the level of lipid peroxidation products returned to normal after about four months in patients who received radiotherapy combined with chemotherapy. The results of our work on antioxidant enzyme activities and lipid peroxidation products level observed six months after brachytherapy were statistically significant. The activities of catalase and glutathione peroxidase and the level of thiobarbituric acid reactive substances in the erythrocytes of the patients returned to normal levels after the end of therapy.

CONCLUSIONS

We conclude that there is no significant change in lipid peroxidation and antioxidant status in the blood of cervical cancer patients directly after brachytherapy treatment. However, our results clearly suggest that there is a change in lipid peroxidation and antioxidant status with reference to a follow-up period of about six months in length. The normalisation of CAT and GSH-Px activity and erythrocytes TBARS level responses to an improvement in the

clinical state of the patients and may be due to the arrest of tumor growth as a result of radiotherapeutic treatment.

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