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Role of superoxide dismutase in individualization of breast cancer radiation therapy protocols

KEYWORDS: Radiotherapy; Clinical Protocols; Breast Neoplasms; Superoxide Dismutase; Tumor Markers, Biological

ABSTRACT

Background: *The goal of this study was to introduce a new predictive biomarker assay that might improve a clinical irradiation treatment of malignant diseases.*
Methods: *Thirty-two peripheral blood samples obtained from breast cancer patients were analyzed for superoxide dismutase (SOD) after irradiation with gamma rays (^{60}Co). SOD was measured in subcellular fractions prepared from unirradiated and irradiated blood samples (McCord and Fridovich). The activity of SOD was measured by the method of Misra and Fridovich and protein concentration by the method of Lowry et al.*
Results: *Antioxidant radiation response of patients' blood cells was very variable and specific for each individual. The results indicated that the radiation response during radiotherapy directly depends on the initial state of antioxidant activity in the blood of cancer patients. In the blood samples with high level of SOD activity the irradiation decreased enzymatic activity while in the samples with medium or low level of SOD, the SOD activity was preserved or increased by irradiation with 2 Gy of gamma rays.*
Conclusion: *We showed that the modulation of SOD activity in blood cells after irradiation in vitro might be used as predictive biomarker in individualization of therapy protocols.*

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INTRODUCTION

The molecular and cellular basis of radiosensitivity is very complex but it would be very important to have a predictive assay for individual estimation of the radiation dose, which would lead to better treatment results. Our research is related to the application of biochemical and cell biology methods in radiation oncology diagnostics, prognostics and therapy.
The enzymes of the antioxidant (AO) defense system represent the key component of the cell defense mechanism and tissue protection system from the harmful effects of free reactive radicals, which influence the genome and other biomolecules in cells (1).
As it is known, the exposure of cells to ionizing radiation leads to formation of the reactive oxygen species (ROS), which are associated with radiation induced cytotoxicity (2). Following the discovery of a group of enzymes - the superoxide dismutases, scavengers of the superoxide radicals, many investigations have been devoted to the role of superoxide radicals in radiation damage, and to the possible protective action of the superoxide dismutases against radiation. The Mn superoxide dismutase (MnSOD) and the CuZn superoxide dismutase (CuZnSOD) are the key intracellular AOs in the metabolism of ROS (3). In most of the previous work it has been reported that MnSOD may play a central role in protecting cells against the ROS injury during the exposure to ionizing radiation. Although MnSOD is clearly involved in radioresistance, there is an obvious need for further investigation of the role of mitochondria oxidative stress in radiation injury (4).

MATERIAL AND METHODS

Thirty-two patients with breast cancer participated in this study. Patients who had signed an informed consent were selected for examination. Whole blood samples were obtained by venipuncture using heparinized syringes and needles. Blood samples were irradiated at room temperature. The radiation dose employed was 2 Gy of ^{60}Co γ-rays, and the dose rate was 0.45 Gy/min. The dimensions of the radiation field were 20 x 20 cm and the distance from the source was 74 cm. The procedure of McCord and Fridovich (5) was used with minor modifications for the subcellular fractionation. The activity of SOD in blood samples was measured by the method of Misra and Fridovich (6). The reaction of autoxidation of epinephrine to adrenochrome was performed in 3 ml of 0.05 M Na_2CO_3 at pH 10.2. Inhibition of autoxidation was monitored at 480 nm. The protein concentration was determined by the method of Lowry et al. (7). The results were expressed in units of enzyme activity. One unit of SOD was defined as the amount of protein that caused 50% inhibition of the conversion rate between the 3rd and 4th minute of incubation.

RESULTS

The specific activity of SOD in blood samples from the first group of patients with low-level enzyme activity (29.3 ± 3.3 U/mg protein) was markedly increased ($p < 0.05$), and found to be 50.9 ± 11.9 U/mg protein, following irradiation with 2 Gy of gamma rays. In the second group of patients average values of SOD activity in blood were 67.9 ± 2 U/mg protein. After dose of 2 Gy of gamma irradiation the activity of SOD in blood samples was decreased (51.9 ± 9.8 U/mg protein). Particularly, in the irradiated blood samples from third group of patients with high level of SOD activity, the irradiation caused the decreasing of enzymatic activity (111.8 ± 9.7 *vs*. 87.2 ± 13.5, $p < 0.05$).

DISCUSSION

There are a lot of clinical and biomedical factors that may predispose patients to the development of complications of radiotherapy (8,9). Understanding the biological response to ionizing radiation requires characterization of the particular bio-molecular system activated by those stimuli, such as antioxidant enzymes (10,11). Our results showed that the high level of SOD activities

may be related with the high susceptibility of blood cells to carcinogenic agents and the response of cells to irradiation is very weak (12,13). Cellular response is initiated by the deposition of energy during passage of radiation through sensitive cellular targets and production of macromolecular damage by ionization events and free radicals. SOD, as a biomolecule, may be damaged by free radicals and the enzymatic activity was significantly decreased (13). Clinical and experimental practice showed that there are dose and time dependent response in radiation effects and regional differences in tissue vulnerability to radiation. In account to that, we could expect harmful effects on the healthy tissues. On the other hand, starting from the low initial level of SOD activity in whole blood may be beneficial in processes such as induction and defense against reactive free radicals producing after radiation with 2 Gy of gamma rays. Nevertheless, these findings may ultimately be useful in devising new strategies to improve the therapeutic ratio in cancer treatment (14,15).

CONCLUSION

Antioxidant radiation response of blood cells from different patients is very different and individually specific. This observation means that the individual radiation response during radiotherapy directly depends on the initial level of antioxidant enzymes or the total antioxidant status (TAS) in the blood of cancer patient. Thus, TAS method could be used as the predictive enzyme assay in clinical setting, with potential to predict and estimate the effects of conventional radiotherapy.

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