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Protective effect of local temporary ischemia depends on applied dose of radiation

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Abstract

The aim of this study was to verify hypothesis that protective effect of local temporary ischemia depends on dose of radiation. 56 male WAG-strain rats were used. Total body irradiation with 3×3 and 3×5 Gy was performed. Local temporary ischemia was induced by clamping the tail base. The biochemical parameters were the thiobarbituric acid-reactive substances (TBA-RS). In bone marrow smears the polychromatic erythrocyte (PCE) numbers were counted and the numbers of micronucleated PCEs were analyzed. In small intestines the numbers of crypts were calculated. The levels of TBA-RS in the serum of the animals irradiated with a 3×3 Gy dose were significantly different ($P<0.002$). Also in animals irradiated with a dose of 3×3 Gy the numbers of intestinal crypts were different ($P<0.05$). In animals irradiated with dose 3×5 Gy, for analyzed parameters differences did not achieve statistical significance. Local temporary ischaemia provides general protection against radiation damage for lower dose. This protective effect disappeared after applications of a higher dose of radiation.
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Keywords: Radiation; Anemia; Ischemic preconditioning; Hypoxia; Lipid peroxidation; TBA-RS

1. Introduction

Since the 1950s it has been widely accepted that the level of oxygen in tissue influences the effects of radiotherapy [1]. The physical relation between oxygen concentration and effectiveness of photon radiation is expressed by what is called the oxygen enhancement ratio [2]. Recognizing hypoxia as one of the crucial problems of radiotherapy is based on the thesis that cells with lower levels of oxygen (hypoxic cells) are more resistant to irradiation. The tumor is recognized as heterogeneous structure with specific vascular network, tumor microenvironment and inadequate lymphatic system. Over the course of the cancer formerly hypoxic cells that survived radiotherapy may exhibit an improvement of oxygen concentration, which enables a quick repopulation of these cells, which survived the radiotherapy what in consequence cause resulting in recurrence and dissemination of the cancer. The model of tissue

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hypoxia described above is static and does not reflect the dynamic character of changes in oxygenation status. According to the principles of physiology, in healthy organisms at rest all tissues are correctly oxygenated. Physical exercise increases oxygen consumption in muscles. In response, the organism must supply more oxygen, resulting in a higher heart rate and higher frequency of respiration. Diseases affecting gas exchange in tissues changes these compensatory mechanisms. The following three clinical situations may serve as examples: anemia (often occurring with cancer) distinguished by a lower number of erythrocytes that causes diminished transport of oxygen to the tissues; respiratory diseases affect gas exchange in lungs and lower oxygen intake; left heart insufficiency makes the myocardium unable to properly circulate blood through the peripheral tissues causing local peripheral ischemia and hypoxia. In all these diseases, the higher amounts of oxygen required during increased physical activity cause elevated oxygen consumption in proximal tissues, leaving cells in the peripheral regions hypoxic. The most common symptoms of hypoxia are dyspnea, fatigue and peripheral cyanosis. Stenocardia and claudication may be also present in these diseases without stenoses in arteries. In the early stages of the above-described diseases patients report occurrence of these symptoms only during exercise, not while at rest. However, the previously mentioned symptoms are present at rest in advanced stages of the diseases. This helps explain why a tumor, like a peripheral tissue, in one situation is well oxygenated and in another is hypoxic. The oxygen enhancement ratio takes into account only stable oxygen levels in irradiated tissues, but it is clear that this is only one of all possible clinical situations related to tissue hypoxia. When we want to take under consideration the entire range of oxygen level fluctuations, the following question arises: Does a temporary hypoxia or ischemia change the effect of radiotherapy? Analysis of data concerning the cell damage during ischemia/reperfusion phenomenon and during radiation shows that in both cases the free radicals are responsible for the damage. In case of hypoxia (what often means the same as ischemia) free radicals are delivered mainly by the neutrophils whereas during irradiation the free radicals are produced by photons reacting with water particles. Since the 1980s it has been known that there exists the phenomenon called ischemic preconditioning. Temporary ischemia increases tissue resistance to the damages inflicted by a total long-term ischaemia [3]. This phenomenon is present in the myocardium, skeletal muscles [3–5], as well as, in the brain, kidneys, lungs, liver and intestine [6–10]. Moreover, there is an evidence that local temporary ischemia produces general resistance to ischemia [11]. If local ischemic preconditioning causes resistance of an organism to ischemia, it should also protect against the effects of irradiation. This hypothesis, earlier verified [12,13], needs further research.

2. Materials and methods

2.1. Animals

Male WAG-strain rats weighing 230–250 g (5–6-months-old) were used in all experiments. The animals were housed four per cage, under controlled temperature (20–22 °C), humidity (60–70%), lighting (12 h light/dark cycle) and provided with food and water ad libitum.

2.2. Experimental protocol

All the procedures have been reviewed by Ethical Committee of the Warsaw Medical University. Total body irradiation was performed with the Philips Co-60 source. Rats were exposed individually in plexi-glass immobilization chambers. The calculated dose rate was 0.595 Gy/min. Local ischemic preconditioning was induced by clamping the tail base (three times for 3 min, with 1 min pause in between). This procedure was also performed on the rats in the individual immobilization chambers immediately before each irradiation. Briefly, pO_2 was measured by needle microelectrode (type 768-20R, Hugo Sachs Elektronik, Germany) implanted subcutaneously in 1/3 proximal length of the tail and connected to Oxygen Partial Pressure Module (Hugo Sachs Elektronik, Germany). Simultaneously, changes in cutaneous microcirculation were monitored using laser Doppler flowmetry (Laser Flowmeter type BRL-100, Germany). Furthermore, in all animals assessment of cutaneous microcirculation was performed to verify clamping-induced tail ischaemia [12,13].