Altered Activity of Respiratory Chain Enzymes in Mitochondria of Peripheral Blood Lymphocytes after Irradiation in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 8, pp. 158-159, August, 1995
Original article submitted October 7, 1994

The effect of ionizing radiation on the activity of succinate dehydrogenase (complex II) and succinate-cytochrome C-oxidoreductase in peripheral blood lymphocytes is studied on rats exposed to whole-body γ-irradiation in doses of 9.5-10.5 Gy. On day 5 after irradiation, when the number of lymphocytes is sharply reduced, enzyme activity in the remaining population is found to be reliably increased. These changes are not related to biological cycles. It is assumed that most of the survivors after high-dose irradiation are the cell populations maintaining a high level of oxidative phosphorylation in mitochondria.

Key Words: *succinate dehydrogenase; ionizing radiation; mitochondria; lymphocytes*

The development of effective means of improving radioresistance and the eventual efficacy of post-radiation therapy require knowledge of the dynamics of molecular shifts in the processes ensuring the vital functions of cells and tissues. Of particular importance are the data on the changes in oxidation-reduction reactions and phosphorylation in mitochondria. Analysis of published findings [4,7] shows that this information can be obtained only by using appropriate models which make it possible not only to assess the nature of the radiobiological effect but also to elucidate the molecular basis providing for the survival of cells and the organism.

The high radiosensitivity of human and animal lymphoid tissue is well known, being manifested in stereotypical quantitative and qualitative shifts appearing after irradiation [10].

In view of the above, it is appropriate to study the specific changes occurring in succinate dehydrogenase (complex II) and succinate-cytochrome C-oxidoreductase activity in peripheral blood lymphocytes of animals exposed to high doses of ionizing radiation.

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MATERIALS AND METHODS

The experiments were performed on random-bred male albino rats weighing 200-220 g. The rats were arbitrarily divided into four groups: groups 1-3 (experiment) each comprised 10 rats and group 4 (control) consisted of 5 animals. Irradiation was performed with a GUBE-3000 apparatus (^{60}Co, 1.45 Gy/min). The absorbed doses were 9.5, 10, and 10.5 Gy for groups 1, 2, and 3, respectively. Blood samples from experimental animals were drawn from the caudal vein one day before and 5 days after irradiation. In the control group blood was sampled at the same times. The activity of the enzymes was evaluated cytochemically [6,9] from the number of formazan granules per lymphocyte (succinate dehydrogenase) and from the Keplow index (succinate-cytochrome C-oxidoreduc-

0007-4888/95/0008-0803$12.50

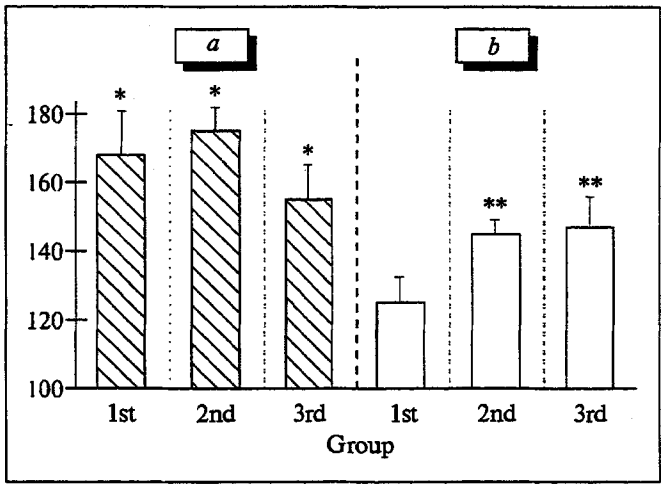

Fig. 1. Changes in succinate dehydrogenase (*a*) and succinate–cytochrome C–oxidoreductase (*b*) activity in peripheral lymphocytes of rats 5 days after irradiation. Ordinate: percentage change in enzyme activity in the experiment vis–a–vis the intact control. $^{*}p<0.01$, $^{**}p<0.05$ in comparison with the control.

tase). The data were processed statistically using the Student *t* test.

RESULTS

The quantitative cytochemical studies reveal no statistically reliable differences between the baseline values within the experimental groups and in comparison with the control. On day 5 after irradiation 90, 80, and 70% of animals were still alive in groups 1, 2, and 3, respectively. At this time, a sharp drop in the number of peripheral leukocytes (from $15\text{-}16\times10^3$ to $8\times10^2\text{-}1\times10^3$) was observed in the experimental animals in comparison with controls. The measurement of enzyme activity in blood leukocytes revealed a reliable increase of the cytochemical indexes in all experimental groups by the 5th day (Fig. 1). It should be emphasized that these shifts are not related to biological cycles, since no changes in the number of leukocytes or enzyme activity were observed in the control group.

These results suggest that following the action of high doses of radiation on lymphoid tissue the cell populations which are preserved are those whose survival and vital functions are maintained by a high level of oxidation-reduction reactions and phosphorylation in mitochondria.

Since succinate-cytochrome C-oxidoreductase is a primary $\Delta\mu_{H+}$-generator [8], the enhanced activity of this enzyme observed by us may be considered to point indirectly to an increased transmembrane difference of electrochemical potential of hydrogen ions on the inner mitochondrial membrane of lymphocytes. Thus, there is every reason to believe that 5 days after irradiation the mitochondrial membrane potential in blood lymphocytes is utilized for ATP synthesis rather than free respiration. This is also confirmed by previous data [2] that no uncoupling of oxidation and phosphorylation in mitochondria was observed in thymocytes irradiated in doses of 6-10 Gy.

Thus, our results and the data on specific changes of oxidation-reduction reactions [1,5] and mitochondrial respiration [3] suggest that prophylactic measures aimed at improving cell and tissue radioresistance must necessarily include factors (drugs) geared toward maintaining the normal functioning of mitochondria and, above all, oxidative phosphorylation.

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