A study of the production of reactive oxygen species by irradiated HEp-2 cells using the dichlorofluorescein a

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The production of reactive oxygen species (ROS) by HEp-2 cells after X-ray irradiation was studied by the dichlorofluorescein assay. 2'7'-Dichlorodihydroluorescein diacetate (50 μM) was added immediately after irradiation, and cells were incubated with the dye at 37oC for 10 min. Then the cells were washed from 2'7'-dichlordihydrofluorescein, and 2'7'-dichlorofluorescein was extracted from cells by treatment with digitonin. The amount of 2'7'-dichlorofluorescein in cells was determined by their fluorescence. Besides, the effect of irradiation on the permeability of cell membrane to 2'7'-dichlorofluorescein was determined. Cells were loaded with 2'7'-dichlorofluorescein by incubation with 20 μM 2'7'-dichlorofluorescein diacetate for 30 min at room temperature. The exit of 2'7'-dichlorofluorescein from control and irradiated cells was determined in a fluorimeter cuvette at 37oC. It was shown that irradiation increased ROS production by HEp-2 cells, but the dose dependence of the effect was distorted. The distortion of the dose dependence was caused by an increase in cell membrane permeability to 2'7'-dichlorofluorescein after irradiation and the exit of 2'7'-dichlorofluorescein from cells.

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