Dose-Rate Effects in Biomembranes

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Dose-rate/membrane/lipid peroxidation

Mice were whole body X-irradiated with different doses and different dose-rates. Directly hereafter lipid peroxidation capacity of the liver membranes was measured. The lag phase prior to rapid peroxidation was clearly shortened at higher doses and also at lower dose rates when the same dose was applied. The dose-rate effects were found at dose-rates less than 1-1.5 Gy/min. With a dose-rate as low as 0.2 Gy/min radiation effects on the liver with doses of 7.5 Gy could be found. The significance of these findings for cell survival after irradiation is discussed.

INTRODUCTION

A major part of biomembranes is made up of lipid molecules. A relation has been found between fatty acid composition and radiosensitivity in fatty acid auxotroph K-1060 strain of E. coli\(^1\)\(^-\)\(^3\). It was observed that an increased number of carbon-carbon double bonds in the fatty acids corresponds with a higher radiosensitivity of the cells and radiation-induced lipid peroxidation was suggested as the underlying mechanism\(^4\). The suggested process of radiation-induced lipid peroxidation in vivo is by far clear at the moment. Conflicting results are found in the literature with respect to the contents of lipid peroxides or products of lipid peroxidation in freshly isolated tissues after in vivo irradiation\(^4\)\(^-\)\(^6\).

Recent experiments performed by us\(^6\) showed that in mouse liver no enhanced level of malondialdehyde could be observed after whole body irradiation with different doses. However, in vitro testing of lipid peroxidation after in vivo X-irradiation showed a clear radiation effect. The absence of an enhanced level of malondialdehyde in the liver after irradiation might be explained by the metabolic breakdown of this product by the liver\(^7\).

Liposomes prepared from phospholipids which extracted from cellular membranes were used to determine the radiosensitivity of the different fatty acids. From these experiments we concluded that especially the polyunsaturated fatty acids docosahexanoic acid (22:6) and arachidonic acid (20:4) were peroxidized in an oxygen dependent and dose-rate dependent process\(^8\). This means that more lipid peroxides were found at a certain dose when the irradiation was performed at a lower dose-rate. Dose-rate dependency for the oxidation of sodium linoleate and soybean lipids
were reported by other investigators\textsuperscript{6,9,10}.

We are not aware of any report in the literature on dose-rate effects measured on biomembranes.

This study shows that after \textit{in vivo} X-irradiation of mice, the lipid peroxidation \textit{capacity} of liver membranes is dose-rate dependent.

\section*{MATERIALS AND METHODS}

C57BL mice of our laboratory were used when they were three to four months of age. Whole body X-irradiation was performed with a Philips Müller MG 300 X-ray machine operated at 200 kV and 15 mA. The beam was filtered with 0.5 mm Cu and 0.5 mm Al; the h.v.l. was 1.1 mm Cu. Different dose rates were obtained by changing the focus-object distance. Immediately after irradiation the mice were killed by cervical dislocation and the livers were taken out. A 10 per cent homogenate was prepared in 0.15 M KCl in a Potter Elvehjem homogenizer with a motor-driven Teflon pestle (clearance 0.20 mm).

The \textit{in vitro} assay system for the measurement of lipid peroxidation \textit{capacity} was performed with a suspension that was shaken in air in an erlenmeyer flask at 37°C. Every 30 minutes a sample was taken, up to three hours; the last sample was taken four hours after the start of the incubation. Lipid peroxide formation was assayed by measuring the concentration of malondialdehyde in the samples with the aid of thiobarbituric acid as described previously\textsuperscript{6,12}.

Protein contents was determined by the method of Lowry \textit{et al.}\textsuperscript{13} Bovine-serum albumin was used as standard.

\section*{RESULTS}

Experiments such as illustrated in Fig. 1, were performed with 25 mice divided in 5 groups of 5 animals each. An X-ray dose of 30 Gy was given to 4 groups at dose rates of 1.2, 0.8, 0.4, and 0.2 Gy/min. As can be seen in Fig. 1, it takes about 2 hours under the experimental conditions used, before the control tissue forms lipid peroxides at a rapid rate (open circles). A shortening of the lag phase prior to rapid lipid peroxidation is found when the homogenates were obtained from \textit{in vivo} irradiated animals. This effect is most pronounced at the low dose-rate. The lag period is progressively shortened with lower dose-rates which means that the lipid peroxidation \textit{capacity} in these tissues is increased with decreasing dose-rates. In the previous experiments\textsuperscript{5} with a dose rate of 1 Gy/min, relatively high doses had to be applied to measure a dose dependency in lipid peroxidation \textit{capacity}. When whole body X-irradiation with low dose-rate is performed, the effect on lipid peroxidation \textit{capacity} can be found at much lower doses than that in Fig. 1. This is illustrated in Fig. 2. A dose of 7.5 Gy already gives an enhanced lipid peroxidation \textit{capacity}. In analogy with the experiments as shown in Fig. 1 a shortening of the lag phase is observed when homogenates from \textit{in vivo} irradiated tissues were incubated. From
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Fig. 1. Formation of the lipid peroxidation product, malondialdehyde (MDA) during incubation of mouse liver. Effect of in vivo irradiation with 30 Gy of X-rays at different dose-rates.

Fig. 2. Dose effects on lipid peroxidation in liver at the relatively low dose-rate of 0.2 Gy/min. Whole body X-irradiation with a dose of 3.75, 7.5 or 15 Gy was performed.
the experiments as shown here, it is concluded that more potential damage to lipids in biomembranes is caused by X-irradiation with a low-dose rate.

DISCUSSION

The lipid peroxidation process in the incubating homogenates is caused by the biomembranes present in these suspensions. The membranes of the endoplasmic reticulum, the microsomes, having the highest peroxidation capacity, while the soluble part of the cell dose not yield lipid peroxides at measurable amounts. These findings on the \textit{in vivo} irradiation effects are consistent with the results of \textit{in vitro} irradiation experiments on subcellular fractions of rat liver as reported by Wills and Wilkinson. Recently we have found a correlation between the length of the lag phase prior to \textit{in vitro} lipid peroxidation and the amount of antioxidant molecules. It seems that some compounds with antioxidant properties play an important role in the process of lipid peroxidation in biomembranes. Vitamin E can very effectively protect polyunsaturated fatty acids against radiation-induced lipid peroxidation \textit{in vitro}.

It is generally known that in most cellular systems increasing dose-rates leads to an enhanced cell killing especially with sparsely ionizing (low LET) irradiation. It is supposed that cells can recover from sublethal damage during irradiation with relatively low dose-rates. The experiments in this paper suggest that lipid peroxidation must be masked in most biological systems. Experiments to examine the effect of different dose-rates on functional activities in biological membranes of cells are now in progress in order to further verify the meaning of the results reported in this communication.

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