The Effect Of Gamma Irradiation On Collagen Fibril Structure

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Abstract—The structure of mouse skin collagen fibrils treated with 500 rad gamma irradiation was studied by electron microscopy. Examination of specimens after 1, 4 and 8 weeks of treatment revealed collagen fibrils consistently larger in diameter than those from normal specimens with the majority of irradiated fibrils measuring 110-138 nm, as opposed to 62-84 nm in the control. No marked differences in cross-linking were detected between irradiated and control reconstituted type I or type III collagen, either by polyacrylamide gel electrophoresis or by thermal stability measurements.

Key words: Electron microscopy, gamma irradiation, collagen structure.

INTRODUCTION

It is now well recognized that gamma irradiation is accompanied by defects in various tissues and organs (Clore et al., 1979; Rojkind and Kershenobich, 1981; Altman and Gerber, 1983; Rennard et al., 1984). Special attention has been focused on collagen and non-collagenous proteins (Vladascu et al., 1976; Drozdz et al., 1982; Nikolaeva et al., 1988; Panizzon et al., 1988; Wegrowski et al., 1988).

Electron microscopy has been used as a tool for detecting any alterations of collagen fibrils brought about by gamma irradiation (Kononenko, 1985; Liu et al., 1989). We have previously found by means of electron microscopy (Tzaphlidou et al., 1991) that 500 rad gamma irradiation leads to aggregation of mouse skin collagen into a regular arrangement of fibrils, as well as to normal axial relationships between the molecules within a fibril.

In the present paper, we report skin collagen abnormalities from in vivo gamma irradiated mouse skin material. Since in skin, type I is associated with type III collagen (Epstein and Munderloh, 1978), modifications in collagen cross-linking in type I and type III collagens after in vitro irradiation are also discussed.

MATERIALS AND METHODS

For in vivo studies, male Swiss albino mice 5 weeks of age were used. Mice were irradiated in a 60Co radiotherapy unit (St. Sabbas Hospital, Athens) with a total dose of 500 rad, allowing irradiation of the whole body, as previously described (Tzaphlidou et al., 1991). After irradiation the animals were kept in groups of four.

Non-irradiated age-matched mice were used as controls. Skin pieces 1 mm² were obtained from each mouse of each group after 1, 4 and 8 weeks of irradiation. The preparative methods culminating in the production of electron micrographs of collagen fibrils were described previously (Tzaphlidou et al., 1991). For measurements of fibril diameters, areas of cross-sectional collagen were photographed. A minimum of 200 collagen fibrils from at least 4 micrographs were analysed for each mouse. Thus, as in each group four mice were involved, the diameters of at least 800 fibrils from 15 micrographs were averaged for each group. The mean and standard deviation, as well as the unpaired t test were calculated with a laboratory computer.

For in vitro studies, native-type collagen fibrils, reconstituted from acetic acid-soluble rat tail type I collagen (Serva Feinbiochemica Co.) and human placenta type III collagen (Sigma Chemical Co.), were prepared as previously (Tzaphlidou et al., 1982). Thermal stability measurements of 500 rad irradiated collagen specimens were carried out as in earlier publications (Tzaphlidou and Chapman, 1984).

Polyacrylamide gel electrophoresis (PAGE) was performed according to Tzaphlidou (1987). Electrophoretograms were scanned with a Hoefer scanning densitometer under identical conditions.

RESULTS

We have previously shown (Tzaphlidou et al., 1991) that in 500 rad gamma irradiated mouse skin collagen fibrils the parallel packing remains throughout without any areas of disorganization. Also all fibrils were seen to retain normal banding periodicity. However, such fibrils revealed a marked increase in their diameter at different times after irradiation, i.e. 1, 4 and 8 weeks. These
I. Leontiou VI (I. et al., 1981) showed that the ultrastructure of collagen fibrils seen in cross section after 1.4 and 8 weeks of 500 rad gamma irradiation. Control fibril diameters (a) are compared with irradiated ones (b, d) after 1, 4 and 8 weeks. Bar = 0.2 μm.

Irradiated wide diameter fibrils were regular in contour (Fig. 1). On measurements performed on over 800 fibrils for each case, we found a range of diameters 114–144 nm (128 ± 15.4) for fibrils after 1 week of irradiation, 107–129 nm (120.2 ± 10.6) and 114–140 nm (126.5 ± 12.8) after 4 and 8 weeks of treatment respectively. The diameters for each instance were significantly (p = 0) larger than those of collagen fibrils of normal skin: 62–84 nm. The highest mean diameter value was obtained after 1 week of irradiation. Comparison of this value with those from the other two irradiated cases revealed a significant difference (p ≤ 0.003). When the mean diameter of all irradiated fibrils (124.2 ± 12.7 nm) was compared with that of the control (72.7 ± 110.9 nm) by the unpaired t test, again a significant difference was found (p = 0). Due to the larger variation in irradiated fibril diameter than that of the control, the standard deviation of the former was greater than the latter.

Although type I is the major component of collagen in the skin, type III is also present in small amounts. Type III has fibrils of smaller diameter (Fleischmajer et al., 1981) and may regulate fibril and fibre bundle diameter (Epstein, 1974; Lapiere et al., 1977). The observed difference in fibril diameter may be therefore a

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**Fig. 1.** Ultrastructure of collagen fibrils seen in cross section. Control fibril diameters (a) are compared with irradiated ones (b, d) after 1, 4 and 8 weeks of 500 rad gamma irradiation respectively. Bar = 0.2 μm.

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**Fig. 2.** Histograms demonstrating the distribution of collagen fibril diameters in 500 rad gamma irradiated mice and in controls. There is a marked shift to the right (fibrils of larger diameter) in the irradiated subject compared with the normal. Dotted: normal, full shading: irradiated.

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**Fig. 3.** Densitometric tracing of electropherograms: reconstituted collagen (a) rat tail type I and (b) human placenta type III. I: untreated collagen, 2, 3, 4: 500 rad gamma irradiated collagen after 1, 4 and 8 weeks of irradiation respectively. i, ii, iii: areas of bands of molecular weight 100, 200 and 600 kDa respectively. (B, bottom; T, top of the electropherogram).
result of the biosynthesis of type III collagen or of a failure of type I fibrils to mature to their normal diameter.

The effect of irradiation on the physicochemical characteristics of type I and type III collagens separately was examined by SDS (sodium dodecyl sulphate) polyacrylamide gel electrophoresis (Fig. 3). For this purpose reconstituted type I rat tail collagen and type I human placenta collagen were used. As observed from the densitometric tracing (Fig. 3a) in samples after 1, 4 and 8 weeks of irradiation, all type collagen bands are increased. A marked increase from 2 to 5 times the control was detected in bands with molecular weight 600 kDa and over. The band of molecular weight 100 kDa showed the least increase. These findings indicate radiation-induced cross-links stabilizing the structure. The above results are supported by measurements of the thermal shrinkage temperature, $T_s$, of reconstituted type I irradiated collagen. Such measurements showed an increase in $T_s$ by 2 C in all cases (Table 1). This demonstrates that treatment of type I collagen with 500 rad irradiation results in a slight increase of structure stability as greater $T_s$ is indicative of increased cross-linking. No considerable differences in type III collagen were noted either by SDS electrophoresis (Fig. 3b) or by thermal stability measurements.

**DISCUSSION**

Previous studies on reconstituted human amnion collagen irradiated with 0.25 Mrad gamma irradiation have shown an increase in collagen fibril diameter (Liu et al., 1989).

In our study, we obtained clear evidence of abnormalities in collagen fibril structure in skin specimens from mice irradiated with 500 rad gamma irradiation. These abnormalities consist of a significant increase in mean diameter at different times after irradiation, i.e. 1, 4 and 8 weeks. As noted earlier, the highest mean diameter value was obtained after 1 week of irradiation with a significant difference of the values from the other two instances. Although Wegrowski et al. (1988) stated that six months after local gamma irradiation of the pig skin and adjacent muscle a fibrotic inflammatory zone was detected, our results cannot suggest that at a later period of 8 weeks such abnormality can be speculated in our whole body irradiated mice. An answer to whether or not the effects on collagen fibril structure produced by gamma irradiation are temporary or not requires further investigation. However, in all fibrils the periodicity and their regular arrangement were preserved (Tzaphlidou et al., 1991) indicating that the increase in fibril diameter did not alter the collagen molecular and fibril architecture.

Polyacrylamide gel electrophoresis of reconstituted type I rat tail collagen showed radiation-induced cross-links while no considerable findings in reconstituted type III human placenta collagen were noted. Formation of cross-links on human tendon collagen after 500–2500 rad irradiation were also quoted by Nikolaeva et al. (1988) while other studies on hepatic collagen (Vladescu et al., 1976) and reconstituted human amnion (Liu et al., 1989) report destruction of intermolecular bonds. However, it is important to note here that in vitro other factors not present in vitro can influence collagen fibril formation such as the non-collagenous extracellular matrix components (Prockop et al., 1976). Therefore, the differences in collagen fibril diameters detected in irradiated mouse skin collagen may be due to defects of the collagen molecule itself or to abnormal metabolism of these components.

In addition, a decrease of total collagen content was found in the skin of 500 rad irradiated rats (Drozdz et al., 1982) while other workers observed an increase in mouse skin collagen biosynthesis (Panizzon et al., 1988) and in content of type I mouse lung collagen (Miller et al., 1986). Also, an increase in collagen content and biosynthesis in irradiated pig skin was reported by Wegrowski et al. (1988). These authors add that the increase in collagen biosynthesis is more pronounced for type III than for type I collagen.

The manner in which the alterations induced by gamma irradiation in collagen cross-linking, content and biosynthesis have the discussed effects on collagen fibril diameter is not yet clear. It appears that they may provide an opportunity to explore mechanisms of protein processing, to understand some of the factors that control collagen fibril formation and to study the functions of gamma irradiation in tissues.

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**REFERENCES**


### Table 1. Measurements of the thermal shrinkage temperature, $T_s$, of reconstituted type I collagen gels, non-irradiated and irradiated with 500 rad gamma irradiation. Higher $T_s$ values indicate increased cross-linking.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Transition temperature $T_s$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-irradiated</td>
<td>57.0</td>
</tr>
<tr>
<td>Irradiated, 1 week after irradiation</td>
<td>56.8</td>
</tr>
<tr>
<td>Irradiated, 4 weeks after irradiation</td>
<td>56.8</td>
</tr>
<tr>
<td>Irradiated, 8 weeks after irradiation</td>
<td>59.0</td>
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