

Radiation as an independent risk factor for atherosclerosis

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INTRODUCTION Cardiovascular disease is a known complication of radiotherapy for many types of cancer, including head and neck, breast, testicular and Hodgkin's disease (1-4). Radiotherapy is a common treatment modality for these types of cancer and about half of the long-term survivors will have received radiotherapy. There is good evidence to identify radiation as an independent risk factor in human vascular disease, in addition to risk factors like hypercholesterolemia, age, diabetes, hypertension, smoking, lack of exercise and stress. Several studies have shown that radiation of the carotid arteries leads to atherosclerosis, which increases the risk of vascular stenosis, thromboembolism and stroke (3,5). However, the mechanisms involved are not fully understood. The aim of this work was to investigate the development and progression of radiation-induced atherosclerosis and to compare this with age-related atherosclerosis.

MATERIALS AND METHODS ApoE^{-/-} mice, which have elevated cholesterol levels and develop spontaneous atherosclerosis with age, were given single radiation doses of 8 or 14 Gy, or 20 x 2 in 4 weeks to the neck region. Age- and sex-matched sham treated controls were included with each schedule. At various times after irradiation, blood samples were taken and the arterial tree was removed for quantitative histological examination of plaque size and phenotype. All lesions were categorized as initial (macrophage rich, without a thick fibrous cap) or advanced lesion (well-defined necrotic/lipid core or thick fibrous cap). The advanced lesions were also examined for the presence of collagen (Sirius Red staining).

RESULTS We first examined the carotid arteries of ApoE^{-/-} mice sacrificed 1 or 4 weeks after irradiation for the presence of fatty streaks. Fatty streaks are the first visible lesions in the development of atherosclerosis and consist of subendothelial accumulation of foam cells (lipid-containing macrophages). One week after treatment, no fatty streaks were found in carotid arteries of irradiated (14 Gy) or control mice. By 4 weeks after 14 Gy, there was a significant increase in the number of arteries with fatty streaks in irradiated versus control mice (4 of 9 vs. 0 of 9; p=0.04). These results show that already within 1 month after a single radiation dose, the formation of early atherosclerotic lesions was initiated. Next, we showed increased numbers of lesions in irradiated ApoE^{-/-} mice, compared with age- and sex-matched controls, at 22-34 weeks after a single dose of 8 or 14 Gy and the more clinically relevant fractionated schedule of 20 x 2 Gy. This was mainly due to an increase in initial, inflammatory lesions (Figure 1). From 30-34 weeks there was also a significant increase in total plaque burden after single dose irradiation.

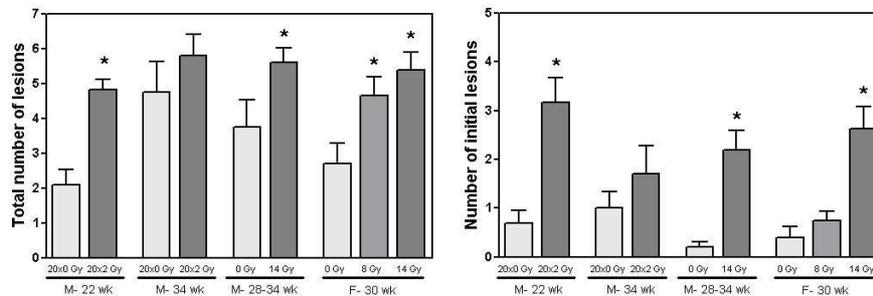


Figure 1. Morphometric analysis of lesions in carotid arteries of ApoE^{-/-} mice. Bar graphs show the mean number of total lesions (left) and the number of initial lesions (right) per animal in males (M) or females (F) after a single dose of 8 or 14 Gy or 20 x 2 Gy. Data represents group means \pm SEM. * $p < 0.05$ compared with age- and sex-matched treated controls.

The most marked difference between irradiated and unirradiated arteries was the inflammatory content and plaque hemorrhage of carotid artery lesions. Granulocyte-rich lesions were common 30-34 weeks after 20 x 2 Gy (100% of carotid arteries), or a single dose of 14 Gy (63% in females, 86% in males), but were found in only 14-33% of age-matched controls. Lesions with erythrocyte-containing macrophages, which is indicative of previous hemorrhage, were seen in 78-90% of the irradiated arteries compared to 21-50% in age-matched controls. Fibrin deposits were seen in lesions of 43-70% of the irradiated arteries, but not in the controls. These inflammatory and thrombotic phenotypes were less evident after a single dose of 8 Gy. The collagen content of advanced plaques in irradiated carotid arteries was also considerably less than in the control arteries (Figure 2). Taken together, these results show that atherosclerotic lesions in irradiated arteries had an inflammatory, thrombotic plaque phenotype with reduced collagen content.

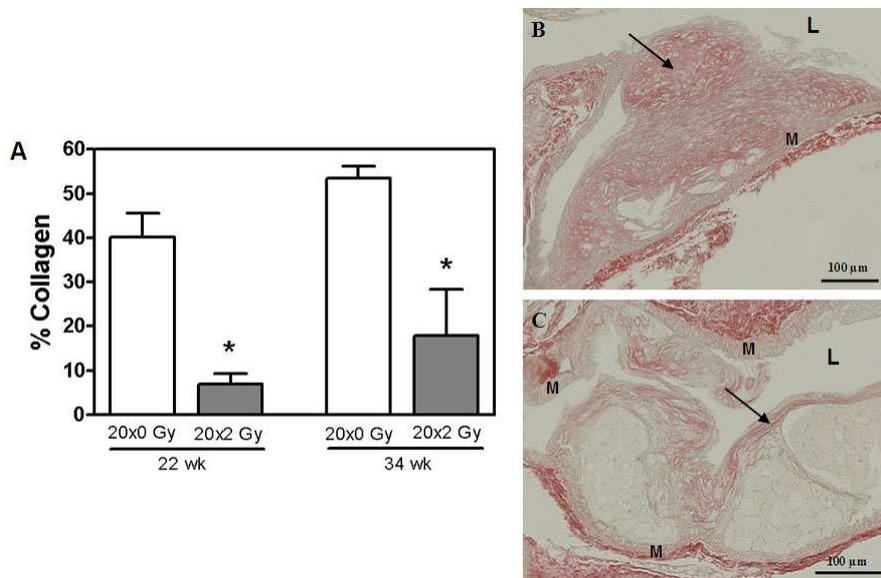


Figure 2. Collagen content of advanced lesions from male ApoE^{-/-} mice at 22 or 34 weeks after 20 x 0 Gy or 20 x 2 Gy (A). Data represents group means ± SEM. * p<0.05 compared with age- and sex-matched treated controls. Representative photomicrographs showing Sirius Red staining (arrows) in advanced lesions at 34 week after 20 x 0 Gy (B) and 20 x 2 Gy (C). L=lumen; M=media.

Analysis of "out-of-field" renal arteries of ApoE^{-/-} mice showed no differences with respect to number of lesions, plaque area and phenotype. Also no increase in systemic markers of inflammation (sICAM-1, sVCAM-1) was seen after local irradiation.

In contrast to ApoE^{-/-} mice, none of the irradiated or control wild-type C57BL/6J mice developed atherosclerotic plaque within the 30-week follow-up period. However, 2 of the 10 irradiated mice developed fatty streaks in the carotid arteries, whereas none of the control mice. This demonstrated the importance of hypercholesterolemia in the development of radiation-induced atherosclerosis, as wild-type mice have much lower levels of total cholesterol and LDL in comparison with ApoE^{-/-} mice.

CONCLUSION We showed that both single doses and fractionated irradiation accelerate the development of atherosclerosis in ApoE^{-/-} mice and predisposed to the formation of an inflammatory, thrombotic plaque phenotype (6,7). Currently we are investigating expression levels of thrombotic and inflammatory markers of endothelial cell damage at early times after irradiation. These results should provide more insight into the mechanism of radiation-induced atherosclerosis and help in the design of effective intervention strategies to prevent the development of atherosclerotic changes in patients following radiation therapy.

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