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LATE EFFECTS OF HEAVY CHARGED PARTICLES ON THE FINE STRUCTURE OF THE MOUSE CORONARY ARTERY

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Correspondence

Late Effects of Heavy Charged Particles on the Fine Structure of the Mouse Coronary Artery.[1]

By

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Running Title: Charged particle and vasculature

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Abstract

Yang, V. V. and Ainsworth, E. J. Late Effects of Heavy Charged Particles on the Fine Structure of the Mouse Coronary Artery.

The ultrastructural changes in the coronary arteries of the B_6CF_1 mouse at 15 months after irradiation are described. Radiation treatment, given at 4 months of age, included single upper-body doses of 1.6 ^{40}Ar Gy, 0.2, 0.4, 0.8, or 3.2 ^{20}Ne Gy, 0.1 or 3.2 ^{12}C Gy and 1.6, 3.2, or 7.0 ^{60}Co-γ Gy. Coronary artery degeneration was found in both irradiated and unirradiated control animals. The major changes included medial smooth muscle degeneration, fibrosis, acccumulation of debris and extracellular matrix. These changes were found to increase with radiation dose and with increasing LET of heavy ions. Based on quantitative analysis, about 12% of the smooth muscle areas examined showed deterioration in the age-matched unirradiated animals. This percentage was increased significantly, ranging between 20% ($P < 0.01$) and 44% ($P < 0.001$), in the irradiated groups. A single dose of 0.2 Gy ^{20}Ne ions induced the significant damages to the smooth muscle cells. The difference of LET dependence for vascular injury in comparison to killing of proliferative cells is discussed.

Key Words: Heavy Charged Particles, Coronary Artery, Smooth Muscle Cells, Quantitative Morphology.

Introduction

Late vascular injury and the mechanism by which it is produced are important as a fundamental radiobiological question. In the past the major emphasis in radiation studies *in vivo* has been placed on rapidly dividing cells, especially those of the hematopoietic system and intestinal epithelium (1). Slowly-turning-over tissues such as vasculatures have received little attention. It has been suggested that radiation-induced vascular damage could be underlying mechanism that contributes to morbidity or potential functional alterations in various tissue (1). The role of vascular damage in the pathogenesis of late radiation morbidity after low doses is unknown. One reason is that there are very few existing model systems by which the late effects on the vasculature can be evaluated quantitatively. From clinical observations after high doses, we know that radiation damage to the vascular system is a contributing factor to degenerative changes in many tissues and can be a significant factor in the patient's late responses to radiotherapy (2-10). In studies on the experimental animals, mural thrombosis occurred in the mouse ventricle at 4 months after 1000 rad local x-rays irradiation, but lesions in the coronary arteries were not observed (11). Studies of radiation effects on the rabbit heart also have been limited to observations of a year or less (4, 9, 10, 12, 13). Relatively few studies have included observations of effects over years after low level partial-body radiation exposures. In recent studies, Yang and Stearner (14-16) used the mouse heart as a model system to evaluate long term effects of low doses radiation on the cardiovasculatures with particular emphasis on high-LET fission neutrons (~ 70 KeV/μm) radiation. Their results showed that the relative biological effectiveness (RBE) was about 3 for high single doses, based on qualitative estimation of medial smooth muscle cells degeneration in the mouse coronary artery at 18 months after

neutron or gamma irradiation. In the same study, the value of RBE estimate increased from 3 to at least 35 - 40 when low fractionated neutron doses were fractionated over several weeks (24 fractions of 0.8 rad given in 23 weeks). They also reported that similar degenerative changes were seen as a consequence of normal aging in the mouse, but radiation, particularly fractionated doses of high LET radiations accelerated this degenerative change.

Increasing activities of humans in space environment will be accompanied by an increased risk of radiation exposure, a small portion of which is high energy heavy charged particles (17). There is less understanding about the late biological effects of heavy charged particles than that of other types of radiation in a space environment. In addition, charged particles are now being used for the treatment of cancer patients (18), and it seems appropriate to obtain information on late effects of heavy charged particles on the cardiovasculature. This study is designed to evaluate quantitatively the late ultrastructural changes in the coronary vessels of the mice after exposure to heavy charged particles, and to compare their effects with those of low-LET radiations.

Materials and Methods

Female B_6CF_1 mice, 4 months old, were given upper body exposures to ^{60}Co-γ radiation or heavy charged particles from BEVALAC according to procedures described in detail elsewhere (19-21). Dose, radiation parameters and sampling times are described in Table I. The animals from which tissues were taken were from an experiment to evaluate Harderian gland carcinogenesis.

At 15 months after irradiation, three to eight animals from each dose group and from age-matched unirradiated group were sacrificed. The hearts were first perfused through the left ventricles with a fixative containing 1% paraformaldehyde, 3% glutaraldehyde, and 0.05% 1,5-difluro, 2,4-dinitro-benzene in 0.1 M cacodylate buffer pH 7.4, then they were removed and cut transversely through the ventricles at the constant site just below the auricles, perpendicular to the long axis. Tissue blocks were washed in 0.1 M cacodylate buffer pH 7.4 and postfixed in OsO_4 for 1 1/2 hours, dehydrated and embedded in Epon 812. One micron sections were stained with uranyl acetate and lead citrate and viewed under Zeiss 10-A electron microscope.

Quantitative data were obtained from 3-4 mice in each irradiated group and from 8 mice in each unirradiated group. Only the coronary arteries at left ventricles were considered in this study. For each animal, a total of six to nine tissue blocks from the three different levels in 2 or 3 large coronary arteries were collected and analyzed. Therefore, for each 3-mouse group a total of 18 - 27 blocks from 6-9 coronary arteries were analyzed quantitatively. The electron micrographs were then taken of each coronary artery in a randomly selected section from each block from which a total of 10-15 sections has been cut. The final magnification for quantitative analysis was 6000x. The numbers of coronary arteries examined are shown in Table I. The fractional volume (V_v) of damaged smooth muscle cells was obtained by the point-counting

method of Weibel (22). In this procedure, a transparent grid was superimposed over electron micrographs of standard magnification and the fractional volume (V_v) derived from the formula $V_v = \frac{P_i}{P_t}$ where P_i is the number of points which overlay damaged areas of smooth muscle cells and P_t equals the total number of points on smooth muscle cells. Statistical analysis was performed where appropriate using the Student's t-test.

Results and Discussion

The coronary arteries from a control animal (Fig. 1) showed a continuous endothelium; the subjacent intimal region contained variable amounts of basal lamina material and was separated from the media by the internal elastica. The medial layers contained smooth muscle cells with basal lamina material surrounding them. Collagen and elastic fibers were present between the smooth muscle cells. Collagen fibers were predominantly present within the adventitial layers. Small areas of smooth muscle cell degeneration were present. The predominant ultrastructural changes after radiation treatments were in the medial smooth muscle cells of the coronary arteries. Smooth muscle cells of a coronary artery after a 0.2 Gy dose of ^{20}Ne showed smooth muscle degeneration, fibrosis and extracellular matrix deposition (Fig. 2). These types of degenerative changes were more severe after higher ^{20}Ne or ^{40}Ar doses. In the group that received a ^{40}Ar dose of 1.6 Gy, the smooth muscle cells were severely damaged and replaced by extracellular matrix materials, debris, secondary lysosomal-like bodies, lipid bodies and fibrosis (Fig. 3). Ultrastructurally, the patterns of these changes were quite similar to those found after fission neutron radiation described by Yang et al. (15). Accumulation of matrix material in the vessel wall after irradiation has been shown to be one of the earliest changes in atherosclerosis (23, 24). The intimal plaques containing aggregates of smooth muscle cells were found in the coronary

arteries after exposure to 0.8 Gy ^{20}Ne and 1.6 Gy ^{40}Ar (Fig. 4). Although the frequency of the plaques was low, none were found in the age-matched control animals. The plaques did not contain lipid bodies which were usually found in atherosclerotic disease. It could not be established if these plaques were the results of direct radiation injury or of some indirect effect, possibly associated with the permeability changes due to the depolymerization of connective tissue membranes (mucopolysaccharides matrix). The loss of integrity of smooth muscle cells in larger coronary arteries after exposure to heavy charged particles and other types of radiation may influence long-term cardiovascular functional capacity and therefore, can increase late radiation morbidity and influence life span (15). Stearner et al. (16) have found that the most severe damage in the myocardium of irradiated mice usually occurred in groups that showed the greatest coronary artery degeneration. The radiation-induced damage in the myocardium and microvasculatures in the mouse heart after exposed to heavy charged particles is also under investigation in our laboratory.

The quantitative data on coronary artery degeneration are shown in Table I. In the unirradiated group, about 12% of the smooth muscle areas examined showed deterioration. In the irradiated groups, this percentage increased significantly, ranging between 20% ($P < 0.01$) and 44% ($P < 0.001$). It should be pointed out that the frequency of degenerated smooth muscle cells after a 0.2 Gy dose of ^{20}Ne ions was significantly higher than that of the control animals. The general relationship appeared to be that the damaged smooth muscle cells increased with radiation dose and with increasing LET, mass or charge of ions. The high-LET ^{40}Ar ions appeared to induce the most severe damage, and ^{60}Co-γ rays induced the least. Based on cell killing *in vitro*

and in vivo, dose and dose averaged LET alone are not sufficient to characterize or predict RBE (25, 26). Particle mass, charge or velocity influence cell killing and the extent to which these factors are also important for characterization of late effects of charged particles remains to be determined. Even in terms of LET alone, the present results showing that stopping ^{40}Ar particles (LET ~600 KeV/μm) are probably more damaging to smooth muscle cells than are stopping ^{12}C (~80 KeV/μm) or ^{20}Ne (~150 keV/μm) departs from the expectation of "overkill" or the wasting of ionization energy at LET greater than 100-200 KeV/μm). At the 10% survival level, the RBE is close to 1.0 for CFU-S harvested from mice exposed to ^{40}Ar particle at the same particle residual range used in the present studies (27). Likewise, based on D_0 for either intestinal microcolonies or testis weight loss, the RBE for ^{40}Ar particles is maximum in the plateau portion of the Bragg curve where the LET is ~100 KeV/μm, and declines with decreasing residual range (and increasing LET) in 4-cm spread Bragg peaks (20, 26). Other emerging results on Harderian gland carcinogenesis following exposure to heavy charged particles show even a higher RBE at 500-700 KeV/μm than at 80-150 KeV/μm. (28). Clearly, the LET-dependence for cell killing and other endpoints is different.

Because the limited data set presented here is inadequate to define shapes of dose-response curves for gamma rays or charged particles, estimates of RBE for smooth muscle damage are imprudent. Our impression, based on the severity of damage and the nature of morphological changes at 0.1, 0.2, or 0.4 Gy of charged particles, is that RBE for charged particles will probably be in the same range as was estimated previously for fission neutrons.

We conclude that a low dose (0.1 - 0.2 Gy) of heavy charged particles accelerates expression of smooth muscle degeneration in the coronary arteries, and that RBE-LET dependence of this injury is different than the relationship

for killing of proliferative cells *in vitro* or *in vivo*. Interactions between radiation-accelerated vascular damage and age associated atherosclerotic changes seems an important area for future investigation to define risk of low doses of high LET radiations.

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Table I Damage of Smooth Muscle Cells of Coronary Arteries in relation to Radiation Doses and Quality

No. of Mice	No. of C.A.* examined	Radiation Parameters	Dose (Gy)	V_v**
4	10	^{40}Ar-570 MeV[a]	1.6	0.43 ± 0.95[c]
3	8		3.2	0.44 ± 0.07[c]
4	9	^{20}Ne-425 MeV[b]	0.8	0.31 ± 0.04[c]
3	7		0.4	0.29 ± 0.03[c]
3	7		0.2	0.20 ± 0.04[d]
3	8		3.2	0.39 ± 0.04[c]
3	7	^{12}C-400 MeV[b]	0.1	0.16 ± 0.02
3	7		7.0	0.35 ± 0.01[c]
3	13	^{60}Co-γ rays	3.2	0.33 ± 0.02[c]
3	13		1.6	0.24 ± 0.01[d]
8	22	Control	0	0.12 ± 0.01

*C.A. = coronary arteries

**V_v = fractional volume of degenerated smooth muscle cells (mean ± S.E.)

a = animals were irradiated at the distal end of the 4 cm spread Bragg peak.

b = animals were irradiated at the distal end of the 10 cm spread Bragg peak.

c = $P < 0.001$

d = $P < 0.01$

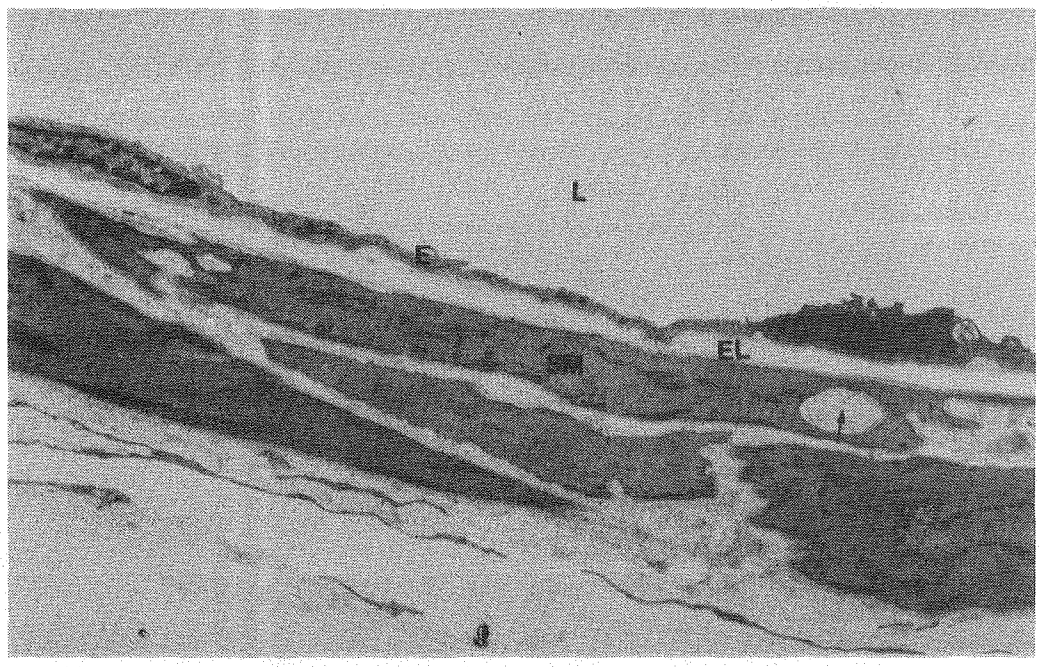

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Figure 1. Electron micrograph of coronary artery from a control animal. Continuous endothelium (E) lining the lumen (L), smooth muscle (SM), elastic lamina (EL) and small area of smooth muscle degeneration (arrow) are noted. x7200

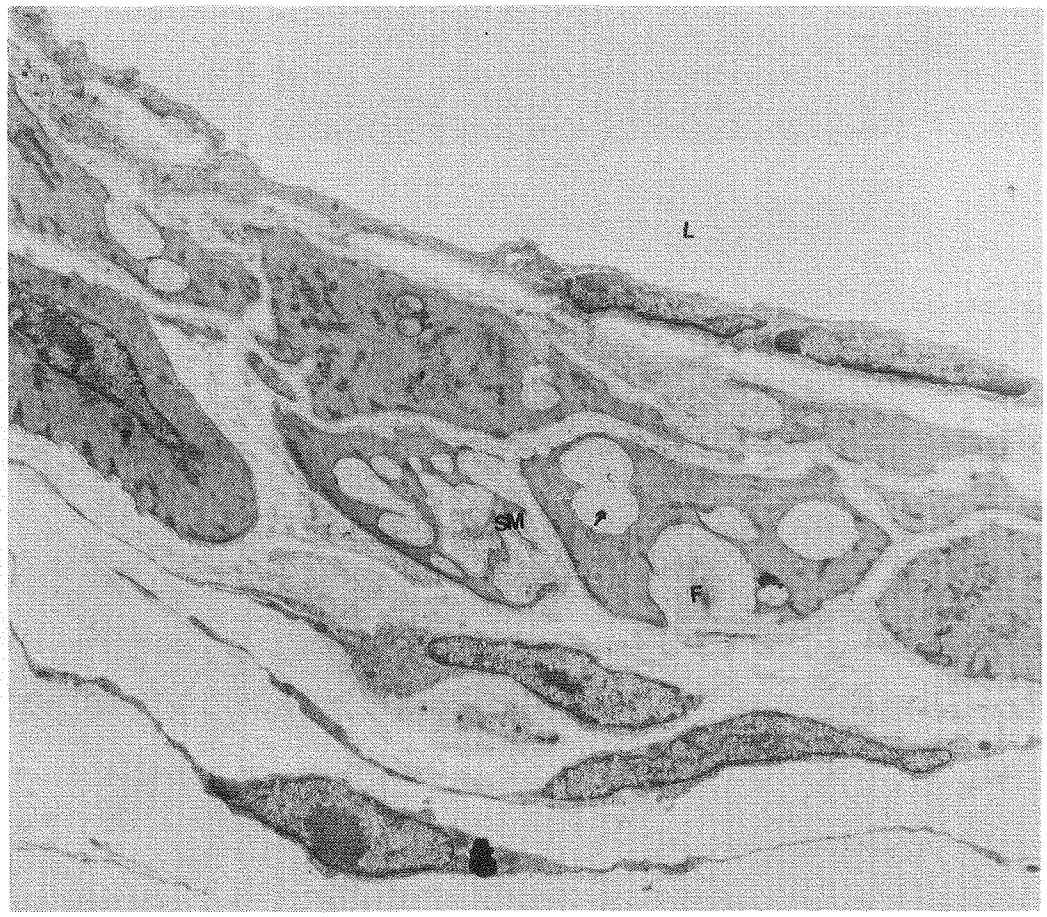

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Figure 2. Electron micrograph of coronary artery after 0.2 Gy ^{20}Ne dose. There is fragmentation of smooth muscle cells (SM) with accumulation of matrix material (arrow) and fibrosis (F). Lumen (L). x7200

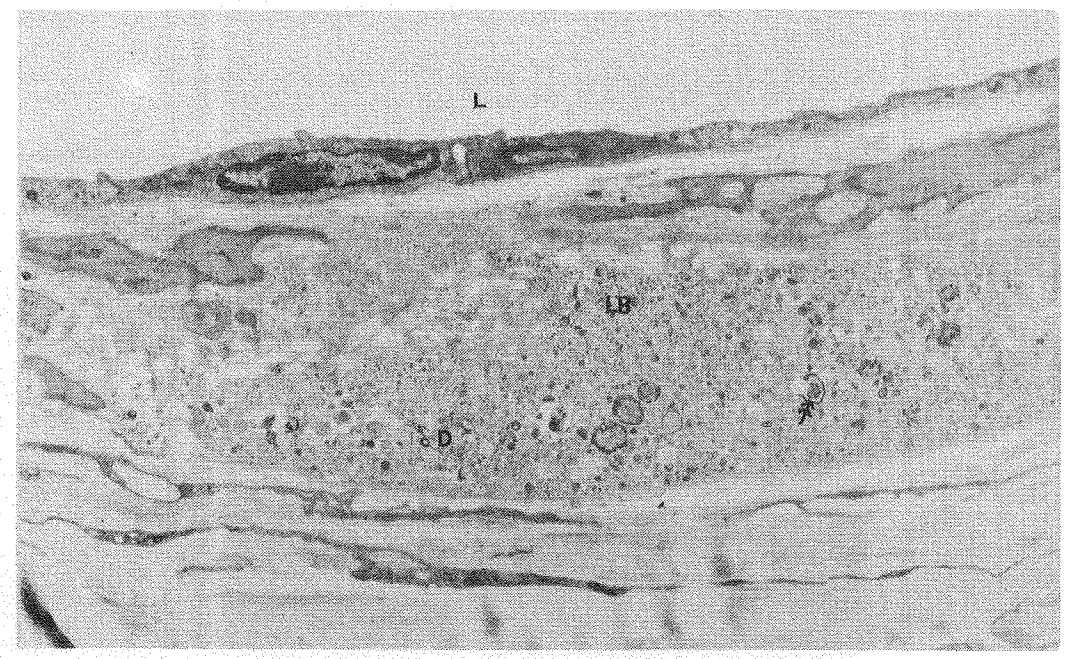

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Figure 3. Electron micrograph of coronary artery after 1.6 Gy ^{40}Ar dose. There is marked loss of medial smooth muscle which is replaced by extracellular matrix material, debris (D), secondary lysosmal-like bodies (double arrow), lipid bodies (LB). Lumen (L). x7200.

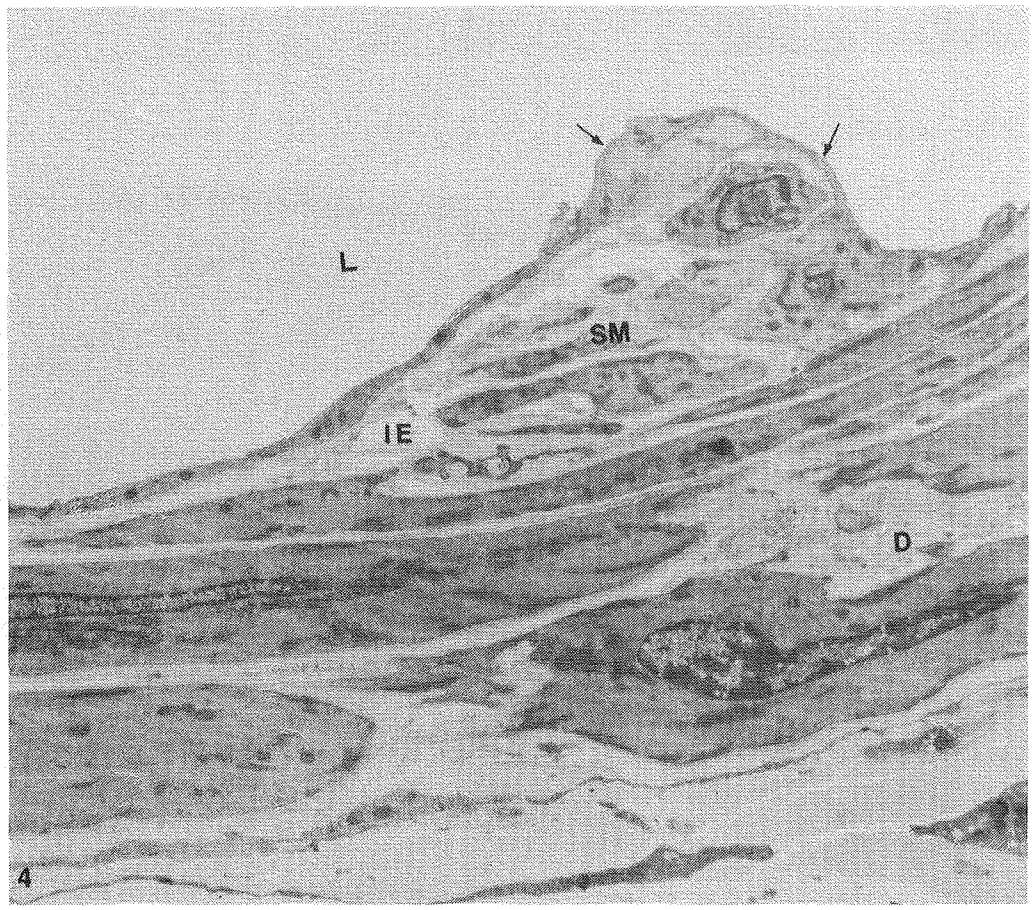

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Figure 4. Electron micrograph of coronary artery after 1.6 Gy ^{40}Ar dose. Arrows mark an intimal plaque. There is fragmentation of the internal elastic lamina (IE), and plaque contains smooth muscle cells (SM). Debris (D). Lumen (L). x7200