Severity of Atherosclerosis in ApoE -/- Mice Following $^{56}$Fe Irradiation is Independent of Plasma Cholesterol Levels

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ABSTRACT

Epidemiological data from atomic bomb survivors, radiotherapy patients, and people with excessive occupational exposure show that radiation exposure increases the risk of myocardial infarction and stroke. Although the character of the radiation in interplanetary space is very different from that encountered on Earth, cosmic radiation might pose a similar risk for astronauts. We established previously that 5Gy $^{600}$ MeV iron ions ($^{56}$Fe), a particularly damaging component of cosmic radiation, exacerbates atherosclerosis in apolipoprotein-E deficient mice. Since the pathogenesis of atherosclerosis involves contributions from a number of risk factors, here we examine the relationship of $^{56}$Fe radiation to plasma cholesterol. In un-irradiated mice, the severity of disease, as measured by average aortic root lesion area, correlated with plasma cholesterol levels. In mice receiving 5Gy $^{56}$Fe, however, lesion size was independent of both total and LDL-cholesterol. This suggests that the effect of $^{56}$Fe may be dominant, overriding some other risk factors.

INTRODUCTION

Radiation is a well-established risk factor for atherosclerosis. For example, radiotherapy for cancer is often limited by the risk of cardiovascular disease. In fact, the benefit of radiotherapy for early breast cancer can be nearly offset by the increased risk of mortality from vascular disease (Early Breast Cancer Trialists' Collaborative Group, 2000). Similarly, after receiving radiation treatments, even relatively young head and neck cancer patients are at significantly elevated risk of stroke (Scott et al., 2009). Exposure to radiation from other terrestrial sources results in similar risk. Atomic bomb survivors had an increased incidence of both coronary artery disease and stroke. Even radiation technologists working before 1950 (when occupational exposure was higher) had increased mortality due to circulatory diseases (Hauptmann et al., 2003). Little is known about the molecular mechanism, however, whereby radiation exposure leads to atherosclerosis years later, so post-exposure therapies to mitigate cardiovascular risk
are lacking. To date, the primary strategy to avoid risk has been to limit exposure.

Astronauts on missions beyond Earth orbit, without the benefit of protection from Earth’s magnetic field, will also be exposed to significant levels of radiation. The character of the radiation to which the astronauts will be exposed, however, is different from that of typical terrestrial forms in that it contains a substantial component of accelerated ions. These ions interact with both tissues and shielding differently than photons such as X-rays or \( \gamma \)-rays. An example is \(^{56}\text{Fe}\), a small but particularly damaging component of galactic cosmic radiation. Recently, we demonstrated that \(^{56}\text{Fe}\) targeted to the aorta and carotid arteries exacerbates atherosclerosis in apolipoprotein-E deficient (apoE \(-/-\)) male mice at a dose 4-8 fold lower that that required for X-rays in the same model. Moreover, the plaques in irradiated mice were more advanced than those in control mice. Such advanced plaques are associated with an increased risk of rupture, which can precipitate myocardial infarction or stroke.

While it is possible to shield astronauts from this type of radiation, shielding will be problematic both because of weight constraints and the thickness of the shielding required. In addition, these ions can interact with shielding materials to produce secondary particles which can exacerbate the damage to tissue. Therefore, some radiation exposure will be inevitable. Since cardiovascular risk factors are often thought to be additive, one strategy to mitigate radiation-induced atherosclerosis might be to control other risk factors. For example, lowering plasma cholesterol might reduce the overall risk of cardiovascular disease and partially compensate for unavoidable radiation exposure.

**MATERIALS AND METHODS**

This question was addressed using apoE \(-/-\) mice, a well-established atherosclerosis model. In this model, although atherosclerosis develops spontaneously, without the need for a special diet or other intervention, both X-rays (Stewart et al., 2006) and \(^{56}\text{Fe}\) (Yu et al., 2011) have been shown to increase the severity of disease. Ten-week old mice were anesthetized with intra-peritoneal injections of 0.15 mg/gm ketamine plus 0.015 mg/gm xylazine, immobilized in chambers developed for this purpose (Yu et al., 2011), and then irradiated with 5 Gy \(^{56}\text{Fe}\) at the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory. Radiation exposure was limited to the upper aorta and carotid arteries by means of a collimator (supplied by NSRL). Control mice were anesthetized, but not loaded into chambers or irradiated. All mice were then shipped to the University of Alabama at Birmingham where they were maintained on a normal diet (Teklad 7917 NIH-31, Harlan Laboratories) for 12-14 weeks. The mice were then euthanized and dissected to assess development of atherosclerosis. Irradiated and control mice were alternated to control for any possible time effects during the two week period.

**Figure 1.** Correlation of total plasma cholesterol with aortic root lesion size. Cross sections of aortic root from un-irradiated control and irradiated (5Gy \(^{56}\text{Fe}\)) mice were stained with Oil red-O and counterstained with hematoxylin. Lesion areas were measured morphometrically under a Zeiss Axiostar Plus microscope using a 1-mm\(^2\) eye-piece grid (100×10,000 \(\mu\text{m}\)\(^2\)) at 100× magnification. Average lesion area for each mouse was then plotted against plasma total cholesterol level. a) For un-irradiated mice, lesion area correlated with plasma cholesterol (\(r = 0.593\), \(p = 0.006\)). For irradiated mice (b), however, this correlation was lost (\(r = 0.0565\), \(p = 0.808\)).
RESULTS AND DISCUSSION

Atherosclerotic lesion size in the aortic root, an atherosclerosis-prone vascular area, was measured by sectioning the root and quantifying cross-sectional lesion area. All mice developed atherosclerotic plaques, regardless of radiation history, as is expected with this mouse model. The average plaque size, however, was significantly increased by radiation, consistent with earlier reports demonstrating a pro-atherogenic effect of $^{56}$Fe (Yu et al., 2011). Average lesion area in mice receiving 5Gy $^{56}$Fe was $23.32 \pm 2.64 \times 10^4 \mu m^2$ as compared to $12.36 \pm 2.07 \times 10^4 \mu m^2$ (mean \pm SEM) for un-irradiated control mice ($p<0.05$ by Mann-Whitney test). As shown in Figure 1a, plaque size strongly correlated with plasma cholesterol levels for un-irradiated mice. For mice receiving 5Gy, however, lesion size was independent of plasma cholesterol (Figure 1b).

The LDL component of plasma cholesterol is considered a strong risk factor for atherosclerosis, while the HDL component is considered protective. Therefore, the relationship between LDL and HDL cholesterol levels with atherosclerotic plaque size was also examined. As shown in Figure 2, while atherosclerotic plaque size correlated strongly with LDL cholesterol levels in un-irradiated mice, the correlation was absent in mice receiving 5Gy $^{56}$Fe. In contrast, as shown in Figure 3, plasma HDL levels showed no correlation with severity of atherosclerotic lesion size.

![Figure 2. Correlation of plasma LDL cholesterol with aortic root lesion size. Aortic roots of control and 5Gy mice were sectioned and stained as in Figure 1, and lesion size was plotted against plasma LDL cholesterol for each mouse. As with total cholesterol, there was a strong correlation with lesion area in un-irradiated control mice (a) ($r = 0.591$, $p = 0.006$), but not in irradiated (5Gy $^{56}$Fe) mice (b) ($r = 0.0206$, $p = 0.993$).](image)

![Figure 3. Lack of correlation of plasma HDL cholesterol with aortic root lesion size. Aortic roots of control and 5Gy mice were sectioned and stained as in Figure 1, and lesion size was plotted against plasma HDL cholesterol for each mouse. HDL cholesterol, a negative risk factor for cardiovascular disease, did not correlate with atherosclerotic lesion size for either control (a) ($r = 0.0683$, $p = 0.775$) or irradiated mice (b) ($r = 0.166$, $p = 0.473$).](image)
plaques, either in irradiated or un-irradiated mice. It should be noted that mice are relatively resistant to radiation. For example, 8 to 14 Gy of x-rays targeted to the major vessels have been shown to accelerate the development of atherosclerotic lesions in apoE-/- mice (Hoving et al., 2008; Stewart et al., 2006), yet epidemiologic studies indicate that radiation can be a risk factor for humans at doses as low as 1Gy (Hauptmann et al., 2003; Ivanov et al., 2001). Since there are no epidemiological data for heavy ions, it is difficult to predict what $^{56}$Fe radiation doses in mice correspond to pro-atherogenic doses in humans. If a similar ratio of susceptibility holds for $^{56}$Fe as for X-rays, however, 0.36-0.63 Gy in humans might pose a similar risk for humans as the 5Gy dose used for mice in this study.

Conventional wisdom holds that atherosclerotic risk factors are additive, and improvement in any one factor should reduce overall risk. For example, even modest reductions in cholesterol levels are thought to reduce the overall risk of clinically significant cardiovascular disease. In this study, the relationship between disease severity and naturally occurring variation in cholesterol levels within the mouse population was examined. Cholesterol levels were not manipulated to rigorously test the hypothesis that lowering cholesterol can protect against radiation-induced atherosclerosis. The results of this study suggest, however, that control of cholesterol may not be effective in ameliorating heavy ion radiation effects on plaque development. As this is a report of a single study using a single animal model with effects at a single radiation dose, however, a definitive answer will depend on further studies. In addition, the time course of loss of the correlation between cholesterol and plaque size is not known, since this is a report of a single time point. Whether other risk factors, such as tobacco use or lack of exercise correlate with severity of $^{56}$Fe radiation-induced atherosclerosis remains unknown. Future studies will be needed to address the possible independence of heavy ion radiation from these and other risk factors.

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REFERENCES


