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Effect of electron radiation on vasomotor function of the left anterior descending coronary artery

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Abstract

The left anterior descending (LAD, interventricular) coronary artery provides the blood supply to the mid-region of the heart and is a major site of vessel stenosis. Changes in LAD function can have major effects on heart function. In this report, we examined the effect of electron simulated solar particle event (eSPE) radiation on LAD function in a porcine animal model. Vasodilatory responses to adenosine diphosphate (ADP; 10^{-9} – 10^{-4} M), bradykinin (BK; 10^{-11} – 10^{-6} M), and sodium nitroprusside (SNP; 10^{-10} – 10^{-4} M) were assessed. The LAD arteries from Control (non-irradiated) and the eSPE (irradiated) animals were isolated and exhibited a similar relaxation response following treatment with either ADP or SNP. In contrast, a significantly reduced relaxation response to BK treatment was observed in the eSPE irradiated group, compared to the control group. These data demonstrate that simulated SPE radiation exposure alters LAD function.

Keywords

solar particle event radiation; left anterior descending artery; endothelium

Introduction

Exploration class missions expected to involve space travel over time periods extending from months to years are being planned by NASA, as well as other space agencies. The space radiation environment exposes astronauts to unique risks from acute and chronic

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exposure to ionizing radiation. Of particular concern is exposure to ionizing radiation emitted during a solar particle event (SPE). In an SPE, magnetic disturbances in specific regions of the Sun result in the release of intense bursts of ionizing radiation, primarily consisting of protons that have a large variable energy spectrum [1, 2]. Especially during space travel missions outside of the protection afforded by the Earth's magnetosphere, the risks from exposure to SPE radiation are a serious concern for astronauts spending extended time in the space environment [2].

Although ionizing radiation has well known effects on vascular endothelial cells [3–10], there is very little information available on the effects of radiation on vascular function, under conditions in which intact animals have been irradiated. The left anterior descending (LAD) artery is an extension of the left coronary artery, descending into the anterior interventricular groove. In most hearts, branches of this artery form anterior septal perforating arteries which enter the septal myocardium to supply the anterior two-thirds of the interventricular septum. The LAD artery and its branches supply most of the interventricular septum; the anterior, lateral, and apical walls of the left ventricle, most of the right and left bundle branches, and the anterior papillary muscle of the bicuspid valve (left ventricle) [11–13]. It also provides collateral circulation to the anterior right ventricle, the posterior part of the interventricular septum, and the posterior descending artery. In a clinical setting, this artery is the most commonly occluded coronary artery [13]. Since the LAD is the major blood supply to the inter-ventricular septum and bundle branches of the conducting system, its occlusion impairs functioning of the conducting system. The result is a “block” of impulse conduction between atria and ventricles known as “right/left bundle branch block” [12].

Further, ionizing radiation exposure increases the incidence of radiation-induced heart disease. This has been observed in the surviving Ukrainian population that was exposed during the Chernobyl nuclear accident [14, 15], as well as survivors of the Hiroshima and Nagasaki atomic bombs [14, 16, 17]. The increased risk may be due in part to the effects of radiation on blood vessels [18]. Radiation-induced heart disease is also recognized in patients receiving radiation therapy [19].

Radiation exposure from SPEs poses a significant health concern for astronauts during missions outside of the Earth's protective magnetic field. SPE radiation doses are expected to result in non-homogenous absorbed doses with large estimated skin doses (predicted by modeling historical SPEs) as high as 32.15 Gy and corresponding doses to the internal blood forming organs, and presumably the heart, of 1.34 Gy [1, 20]. There are no reports concerning the effect(s) of SPE-like radiation on heart function.

In the current report, as a part of a large study utilizing the minipig model to investigate multi-organ effects of SPE radiation (including the skin, hematopoietic system, and immune system), we have analyzed the effects of electron simulated SPE (eSPE) radiation on the vasomotor function of the LAD.

Materials & Methods

Animals

Male, Yucatan minipigs aged 8–14 weeks were obtained from Sinclair BioResources (Columbia, MO). The animals were housed individually with *ad lib* access to water and fed twice daily with standard minipig chow. Animals were given daily enrichment activities and randomly placed in treatment groups, non-irradiated, control (CON) and irradiated (eSPE) animals. All animals and procedures carried out in this study were conducted under protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC). The sample sizes were as follows: Control, Intact = 4; Irradiated, Intact = 3; Control, Denuded = 4; Irradiated, Denuded = 2 for the ADP & BK responses and 3 for the SNP response.

Irradiation

Non-anesthetized animals were placed in rectangular plexiglass cages [75 cm (L) × 32.5 cm (H) × 30 cm (D); 0.5 cm thick walls], containing Napa Nectar hydrating gel. The animals were irradiated with 6 MeV electrons produced by a Clinac iX linear accelerator (Varian Medical Systems) located in the Perelman Center for Advanced Medicine, University of Pennsylvania, at a source-to-skin distance of 5 m, as described by previously by Wilson et al. [21]. The cages were rotated 180° every quarter dose and surface patient dosimetry verification devices (OneDose, Sixel Technologies, Morrisville, NC) were used to confirm the skin doses received. The entire radiation chamber with animals was irradiated with a prescribed skin dose of 25 Gy with 6 MeV electrons over a 3 hour exposure period. Non-irradiated controls (Control group) were placed in the irradiation chamber for the same time period as the irradiated animals, but with no radiation exposure. The energy of electrons was chosen to mimic the September 1989 SPE, as previously described [20]. Under these conditions, the mean dose to the heart was calculated to be 0.35 Gy using a Monte Carlo simulation model developed in a recent study by Diffenderfer et al. [22]. That study describes in detail the development and validation of a Monte Carlo radiation transport simulation of the electron beam. The simulation incorporates computed tomography (CT) scans of the Yucatan minipigs and produces 3D dose distributions in the mini-pig CT geometry. Observations of the minipigs during irradiation concluded that the animals spent 70% of the irradiation time in a prone position and the remaining 30% of the time in a left-right decubitus position. These observations were incorporated into the simulations by adjusting the simulated beam angles to reproduce the average mini-pig orientation during irradiation. The heart volume was digitized within the CT geometry using the Radiation Oncology treatment planning software Eclipse (Varian Medical Systems) and the mean dose to the heart volume was calculated using the 3D dose distribution.

Heart dissection

Animals were humanely euthanized at 30 days after the radiation exposure. As mentioned above, this study was one part of a multi-organ evaluation of the effects of SPE radiation exposure. Therefore, the 30 day endpoint was pre-determined to investigate the acute risk of SPE radiation exposure. The hearts of each animal were removed and immediately placed in ice cold perfusion solution [3 mM MOPS (pH 7.4), 5 mM Glucose, 2 mM CaCl₂, 4.7 mM

KCl, 145 mM NaCl, 1.2 mM NaPO₄, 1 mM MgSO₄, 2 mM Pyruvate]. The entire heart in buffer and on ice was shipped to the University of Missouri for *in vitro* assessment of LAD vasomotor function..

Upon receiving the hearts (within 24 hours of removal), the LAD was dissected and subsequently trimmed of connective tissue and fat. The LAD was segmented into rings, and their outer diameter, inner diameter, and axial length were measured with an Olympus SZH video microscope which was connected to a Spot Insight camera (model 3.2.0., Diagnostic Instruments, Inc., Sterling Heights, MI). Then, the rings' morphological characteristics (axial length, outer and inner diameters) were measured using Image J software (1.34n, NIH, USA).

In Vitro Assessment of Coronary Vasomotor Function

As previously described [23, 24], two LAD rings were mounted on myographs (Globaltown Microtech., Inc., Sarasota, FL) and diameter was adjusted to elicit a maximal response. Briefly, endothelium-dependent, dose-dependent vasorelaxation was assessed in two rings using cumulative addition of adenosine diphosphate (ADP; 10^{-9} – 10^{-4} M) and bradykinin (BK; 10^{-11} – 10^{-6} M); whilst the assessment of endothelium-independent vasorelaxation utilized increasing doses of sodium nitroprusside (SNP; 10^{-10} – 10^{-4} M). The LAD rings were precontracted with prostaglandin F₂ (PGF₂, 30 μ M) and allowed to achieve a plateau in tension development before the addition of vasodilators. PGF contraction is observed for constancy and once this is achieved, the dose response curves for the dilating agents are examined. For each heart ring one was left untreated (intact). The endothelium was removed from ring two; “denuded” by gentle rubbing of the luminal surface with fine-tipped forceps to study the endothelium-independent mechanisms, explicitly smooth muscle relaxation [24]. Ring two was considered to be properly denuded by the inability of BK to induce vasodilation (i.e. less than 5%) in PGF₂ precontracted rings [24]. The order of agonists throughout the entire study was ADP, BK, and SNP. Following each agonist-induced dose response, Krebs bicarbonate solution was replaced at 20 min intervals until resting tension of all arterial rings was reached (60 min), before the next protocol was initiated.

Contractile responses to PGF₂ were compared between the Control, Intact; Control, Denuded; Irradiated, Intact; and Irradiated, Denuded groups using one-way ANOVA. Dose-response curves were analyzed by repeated measures ANOVA. Contrast tests were used to compare each dose between groups (SuperAnova, Abacus Concepts, Berkeley, CA). Reported EC₅₀ are expressed as the mean $\pm$ SEM.

Results & Discussion

Cardiovascular mortality after radiotherapy or accidental radiation exposure has been documented. At a mean heart dose of 1.6 Gy, 94 cardiovascular deaths were reported amongst 363 patients [25]. Current research on radiation-induced cardiovascular disease, with a range of doses to the heart, is necessary. In the present study, the mean heart dose (and LAD dose) measured from Monte Carlo simulations were calculated to be 0.35 Gy. The current study demonstrates that ionizing radiation has a direct effect on the cardiac vasculature.

PGF₂ was utilized to evoke precontraction of the rings. Maximal contractile tensions stimulated by 30 μ M PGF₂ were not different (in a statistically significant manner) in vessel segments isolated from the Control and Irradiated groups: Control, Intact = 12.1 $\pm$ 2.2 g; Control, Denuded = 7.9 $\pm$ 2.0 g; Irradiated, Intact = 7.2 $\pm$ 1.6 g; Irradiated, Denuded = 4.3 $\pm$ 2.1 g.

To investigate the effect of SPE radiation on LAD function, we determined the responsiveness of the isolated vessels to the vasodilators, ADP, BK, or SNP. In response to ADP, intact rings exhibited a similar relaxation response when the results from the eSPE irradiated animals were compared to those from the Control animals. However, denuded vessels from eSPE irradiated animals exhibited a greater relaxation response to ADP compared to the Control group (Fig. 1A). This difference in sensitivity was reflected in the significantly greater EC₅₀ value for the Control, Denuded compared to the Irradiated, Denuded rings (log M: -4.5 $\pm$ 0.2 and -5.5 $\pm$ 0.3, respectively, $p=0.018$). The ADP-induced relaxation in denuded LADs (Fig. 1A) indicates that receptors located in vascular smooth muscle of LAD rings also must be responsive. These data suggest that ADP has both endothelium dependent responses and responses mediated directly on the smooth muscle. In fact, the sensitivity in the irradiated rings suggests that the radiation exposure may activate or potentiate signaling mechanisms more than in the Control rings (Intact or Denuded), in response to ADP. The vasorelaxation on the smooth muscle may involve adenosine release and activation of adenosine A_{2A} receptors [26]. ADP can also induce vasodilation via nucleoside-selective P_{2Y1} receptors ("purinoceptors") located on endothelial cells of porcine coronary arteries [26]. The loss of response to ADP in the Denuded rings may partly reflect a reduced responsiveness of the smooth muscle cell layer to endothelium-derived vasodilators.

In response to BK, an endogenous vasodilator peptide, intact vessels exhibited a significantly greater relaxation response (~ 20%) in the Control group, compared to the eSPE group. The EC₅₀ values for the intact vessels were not significantly different ($P=0.996$) between the irradiated and Control groups. The denuded rings from both the Control and eSPE groups were unresponsive to BK treatment (Fig. 1B), confirming that the BK response is mediated via receptors present on the surface of endothelial cells [27]. The fact that the relaxation response was lower in the radiation-treated group suggests that radiation exposure damages the vascular endothelium. This is consistent with previous results demonstrating that aortic endothelial-dependent vasorelaxation was impaired in rats exposed to a single whole body dose of either 0.5 Gy cesium-137 gamma-irradiation [7] or 1 Gy iron-ion irradiation [4]. BK is an endothelium-dependent dilator that acts directly on endothelial cells, causing the release of nitric oxide (NO), PGI₂ and perhaps endothelium derived dilating factor(s) [28–30]. These factors signal relaxation of the vascular endothelium.

In response to SNP, intact vessels exhibited a similar relaxation response when the results from the eSPE and Control groups were compared (Fig. 1C). The EC₅₀ values were not significantly different between the Control, Intact and the Irradiated, Intact groups, -6.2 $\pm$ 0.1 and -5.9 $\pm$ 0.1 log M, respectively. Removal of the vascular endothelium (denuded) had no effect on the SNP response, consistent with fact that SNP causes relaxation of arterial

vascular smooth muscle. The EC₅₀ values for the Control, Denuded ($-6.28 \pm 0.1 \log M$) and the Irradiated, Denuded ($-6.2 \pm 0.04 \log M$) were not significantly different. SNP generates NO, which is the active mediator responsible for the direct vasodilation in vascular smooth muscle [23, 27, 31]. SNP is an endothelium-independent relaxant agent, so it is not surprising that the isolated rings (intact or denuded) from the irradiated group had similar relaxation responses, compared to the Control group.

The arterial response to the different vasodilators calls into question the effects of radiation in terms of direct endothelial or smooth muscle cell damage. It is clear that with BK treatment, radiation exposure diminishes the arterial relaxation response, suggesting direct endothelial damage. This is consistent with previous observations of altered endothelium-dependent vasodilation in response to radiation exposure [32–35], suggesting an impaired NO-associated mechanism. In contrast to BK, SNP activates endothelium-independent vasodilator pathways. The fact that SNP had virtually no effect between the Intact vs. Denuded rings or the Control vs. Irradiated rings suggests that the smooth muscle is not affected by radiation exposure. However, when treated with ADP the response of the artery suggests sensitivity to both radiation and the removal of endothelium, suggesting a direct effect on vascular smooth muscle and vascular endothelial components. We hypothesize that the contractile response to ADP in the irradiated arteries may be mediated by endothelium derived contracting substances.

In this study, only male minipigs were utilized. It is hypothesized that the radiation-induced effects reported here on LAD function could be greater in female subjects. It has been reported that estrogen treatment of human endothelial cells results in rapid increases of NO via estrogen receptor-dependent activation of endothelial nitric oxide synthase (eNOS) [36]. Further, eNOS expression levels in the aortas of female rats were reported to be significantly greater than in male rats [37]. Further, it is well documented that hormonal replacement therapy may display different effects on contractile functions in coronary vessels [38, 39]. If indeed, ionizing radiation-induced endothelial dysfunction is dependent on NO/eNOS regulation, then the female population would be at a greater risk for cardiovascular complications; therefore, mechanistic studies for radiation-induced endothelial relaxant responses are warranted.

In conclusion, these results imply that alterations in vasoregulation are a consequence of radiation exposure. Our findings suggest that astronauts exposed to a high dose of SPE radiation (or even small doses of radiation to the heart) may be at risk for cardiovascular damage. While the skin dose was high in the studies reported here, the dose to the heart was relatively low (0.35 Gy). Breast cancer radiotherapy doses to the heart are estimated between 0.64 Gy – 2.17 Gy [40]. Further, documented, historically large SPEs have been used to estimate doses to the blood forming organs and presumably the heart (up to 0.46 Gy) [1], which are estimated to be larger than that calculated in this study. The heart dose calculated in this study is lower than the dose of gamma radiation (0.5–5 Gy) previously shown to affect endothelial dependent relaxation in rat aorta [5, 6] and the results reported here indicate that such small doses to the heart pose a cardiovascular radiation risk. Future work will be required to determine the signaling pathways (i.e., NO-dependent vs. –

independent mechanism) involved in SPE radiation-induced blunted relaxation of the LAD artery.

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Figure 1.

Concentration-response curves for adenosine diphosphate (ADP) (panel A), bradykinin (BK) (panel B), and sodium nitroprusside (SNP) (panel C) -induced relaxation of isolated LAD rings from control (non-irradiated) and irradiated pigs. Data are presented as the average $\pm$ SEM. The sample sizes were as follows: Control, Intact = 4; Irradiated, Intact = 3; Control, Denuded = 4; Irradiated, Denuded = 2 for the ADP & BK responses and 3 for the SNP response. Statistical analyses was performed where $n \geq 3$ using the unpaired Student's T

test. A value of $p < 0.05$ was considered statistically significant and denoted by *, when comparing Control, Intact vs. Irradiated, Intact (B).