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10 GY TOTAL BODY IRRADIATION INCREASES RISK OF CORONARY SCLEROSIS, DEGENERATION OF HEART STRUCTURE AND FUNCTION IN A RAT MODEL

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Abstract

Purpose—To determine the impact of 10 Gy total body irradiation (TBI) or local thorax irradiation, a dose relevant to a radiological terrorist threat, on lipid and liver profile, coronary microvasculature and ventricular function.

Materials and methods—WAG/RijCmcr rats received 10 Gy TBI followed by bone marrow transplantation, or 10 Gy local thorax irradiation. Age-matched, non-irradiated rats served as controls. The lipid profile and liver enzymes, coronary vessel morphology, nitric oxide synthase (NOS) isoforms, protease activated receptor (PAR)-1 expression and fibrinogen levels were compared. Two dimensional strain echocardiography assessed global radial and circumferential strain on the heart.

Results—TBI resulted in a sustained increase in total and low density lipoprotein (LDL) cholesterol (190±8 vs. 58±6; 82±8 vs. 13±3 mg/dL, respectively). The density of small coronary arterioles was decreased by 32%. Histology revealed complete blockage of some vessels while cardiomyocytes remained normal. TBI resulted in cellular peri-arterial fibrosis whereas control hearts had symmetrical penetrating vessels with less collagen and fibroblasts. TBI resulted in a 32±4% and 28±3% decrease in endothelial NOS and inducible NOS protein respectively, and a 21±4% and 35±5% increase in fibrinogen and PAR-1 protein respectively, after 120 days. TBI reduced radial strain (19

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± 8 vs. $46 \pm 7\%$) and circumferential strain (-8 ± 3 vs. $-15 \pm 3\%$) compared to controls. Thorax-only irradiation produced no changes over the same time frame.

Conclusions—TBI with 10 Gy, a dose relevant to radiological terrorist threats, worsened lipid profile, injured coronary microvasculature, altered endothelial physiology and myocardial mechanics. These changes were not manifest with local thorax irradiation. Non-thoracic circulating factors may be promoting radiation-induced injury to the heart.

Keywords

vascular sclerosis; blood lipids; ventricular function; total body irradiation; thorax only irradiation; morphology; cardiovascular risk factors

Introduction

The extent to which total body irradiation (TBI), from a radiation accident or radiological terrorism, affects the cardiovascular system is poorly understood. Evidence that TBI-induced injury may be a cardiovascular risk factor comes from longitudinal studies of Japanese atomic bomb survivors. In this population, mortality from cardiovascular disease is significantly increased after more than 40 years after a single dose exposure of 1–2 Gy to the entire body (Preston et al. 2003, Yamada et al. 2004, McGale and Darby 2005).

Radiation therapy has also been linked to the development of cardiovascular disease. For example, radiation therapy for the treatment of peptic ulcers is correlated with an increased mortality from coronary heart disease (Carr et al. 2005). Radiotherapy for breast cancer and Hodgkin's lymphoma is also associated with increased risk of cardiovascular disease (Hooning et al. 2007) and myocardial infarction (Swerdlow et al. 2007), respectively. In these clinical exposures the heart or at least part of the heart was irradiated.

Further evidence that radiation causes cardiovascular disease comes from animal studies. Irradiation of the thorax or specifically the heart in animals induced injury (Cilliers et al. 1989, Fajardo and Stewart 1970, Lauk et al. 1985, Yeung and Hopewell 1985, Yeung et al. 1989) following doses of ≥ 15 Gy. Single dose exposure to 15–60 Gy produced long term effects on cardiovascular function in the rat, resulting in morphological degeneration, mechanical dysfunction (Wondergem et al. 1991), damage to the endothelium (Boerma et al. 2004), and increased mortality (Lauk et al. 1985).

The extent to which exposure to 10 Gy TBI, a potentially survivable dose in a radiation accident or radiological terrorism event (Coleman et al. 2003), results in injury to the cardiovascular system is unknown. In this setting, the entire body rather than a single organ, such as the heart, would be exposed. In such a radiological terrorism event, children would likely account for a significant portion of the population affected. Children may be more susceptible to injury from radiation and certainly the long-term consequences of late effects in individuals exposed as children is greater than in adults and this specifically applies to the heart (Shankar et al. 2008). Thus studies are needed to determine whether a single dose exposure to 10 Gy TBI in the child would injure the cardiovascular system.

Evidence from the clinical use of radiation to treat children with cancer supports the induction of cardiac injury. Exposure to 10 Gy TBI, in the setting of bone marrow transplantation in children treated for leukemia, results in immediate and delayed cardiac abnormalities manifest as decreased left ventricular ejection fraction and abnormal electrocardiogram (Eames et al. 1997, Pihkala et al. 1994). This supports the notion that radiation exposure in children can lead to cardiac dysfunction during development into adulthood. However, these patients with

leukemia also receive chemotherapy which may also contribute to the overall treatment-induced cardiotoxicity.

The confounding effects of chemotherapy on radiation-induced injury can be eliminated using an animal model of radiation injury. A rat model allowing survival following TBI is available (Moulder and Fish 1989) to study radiation-induced injury in vital organs such as the heart at a dose relevant to the radiological terror threat. It is hypothesized that a single TBI dose of 10 Gy would increase circulating lipids resulting in damage to the coronary vasculature and heart function.

The development of cardiovascular disease following TBI may result from direct or indirect causes. For instance, cardiac dysfunction may result from the direct irradiation of the heart during both TBI and local thorax irradiation. Another possibility is that cardiac dysfunction results from indirect injury in which cardiac dysfunction is only manifest following TBI and not irradiation of the thorax alone. The objectives of the present study were to determine the effect of 10 Gy, either as local thorax irradiation or TBI on lipid and liver profile, coronary vasculature and cardiac function in a rat model, and to determine if injury is a result of direct or indirect effects on the heart.

Methods and materials

Experimental animals

Young WAG/RijCmcr male rats (n=62), 5 weeks of age, weight 71 ± 3 g, (41% of that of young adult rats, 8–10 weeks of age, of the same strain) were maintained on sterilized rat chow in a moderate-security barrier facility at the Biomedical Resource Center of the Medical College of Wisconsin (MCW), Milwaukee, Wisconsin, USA. Animal care was in accordance with NIH guidelines. The Animal Care and Use Committee at the MCW approved all protocols.

TBI and thorax only irradiation

Rats received TBI or local thorax irradiation with a single dose of 10.0 Gy. TBI was done with a posterior-anterior field at a dose rate of 1.95 Gy/min. Thorax-only irradiation was done with parallel-opposed lateral fields at a dose rate of 1.62 Gy/min. Irradiation was with 300 kVp orthovoltage x-rays, half value layer to 1.4 mm Cu. The radiation dosimetry has been described in detail previously (Cohen et al. 2007). The TBI dose is low enough so that long-term *in vivo* studies could be carried out following bone marrow transplantation without the risk of acute radiation injury to other organ systems (Moulder and Fish 1989). Non-irradiated rats, not receiving a bone marrow transplant, served as controls. Rats recovered in the barrier facility.

Bone marrow transplantation

One to 2 hr after TBI, rats were given fresh isogenic bone marrow cells using techniques previously published (Moulder and Fish 1989). Rats exposed to local thorax irradiation did not receive a bone marrow transplant nor did the separate un-irradiated controls for this study.

Lipid profile and liver function

Serum was taken at the time of irradiation and then at 20, 40, 60, 80, 100 and 120 days after TBI or after 60, 120, 180 and 240 days after local thoracic irradiation and analyzed for lipid levels (total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides) and liver function (alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) enzyme activity, and total bilirubin, albumin and total protein) (Marshfield Veterinary Laboratories, Marshfield, Wisconsin, USA).

Kidney function

Serum was taken at the time of irradiation and at 120 days after TBI and was analyzed for urea levels (Datta et al. 1999).

Coronary vessel imaging

After 120 days, the hearts of 6 TBI and control rats were isolated and perfused in a retrograde manner at 37°C with bicarbonate buffer (Baker et al. 2008) containing high potassium (16 mM) and low calcium (0.8 mM) at constant pressure (85 mmHg) for two minutes to wash out blood from the coronary vasculature and arrest the heart in diastole. Each heart was then perfused with contrast media and processed for micro-computed tomography as previously described (Karau et al. 2001). The density of coronary vessels was then assessed in hearts from irradiated and non-irradiated animals.

Histology

To evaluate tissue damage at 120 days following irradiation, the entire heart (n=3) was fixed in formalin (10% v/v), embedded in paraffin, and 5µm-thick sections cut from each block and stained with hematoxylin and eosin (H&E) or Masson-trichrome according to standard methods. Ten sections from each heart were evaluated. Hearts from age-matched controls (n=3) were prepared in the same way.

Blood pressure

Systolic blood pressure was also measured non-invasively after 120 days in control and irradiated rats (n=6/group) using a tail cuff plethysmograph (Life Science Instruments, Woodland Hills, California, USA) in conscious rats.

Cardiac echocardiography

Segmental myocardial viability was assessed using two dimensional strain echocardiography in control and irradiated rats (n=6/group) after 120 days. The operator was blinded to identity of the rat. An echocardiograph Vivid 7 (General Electric, Waukesha, Wisconsin, USA) was used with a M12L (11-MHz) linear-array transducer. Closed-chest imaging was performed in the short-axis view at the mid-LV level verified by the presence of prominent papillary muscles. The image depth was 2.5 cm, with 236 frames/second acquisition, second harmonic imaging, and with electrocardiographic gating (Migrino et al. 2007).

Echocardiography image analysis

The images were processed on a workstation with EchoPAC Q analysis software (General Electric). A cardiac cycle was defined from the peak of one R wave to the peak of the following R wave. The method has been previously described (Migrino et al. 2007). The endocardial border was traced in an end-systolic frame. The software automatically selected 6 equidistant tissue-tracking regions of interest in the myocardium. The outer border was adjusted to approximate the epicardial border. The software then provided a profile of radial (myocardial deformation toward the center) and circumferential (myocardial deformation along the curvature) strain (%) with time. End systolic radial and circumferential strain was obtained for each of the 6 segments and the global strain calculated as the average. Three consecutive heart beats were measured and the average used for analysis.

Western blot analysis

The vasculature needs to play an important role in maintaining vasodilation and the balance between pro- and anti-coagulation. Endothelial (e) nitric oxide synthase (NOS) and inducible (i)NOS are responsible for producing nitric oxide, a critical mediator of vasodilation. Protease

activated receptor (PAR)-1, the vasculature receptor for thrombin is responsible for mediating coagulation. The effects of radiation on these mediators of vascular function were assessed. In three irradiated and control animals, the free wall of the left ventricle was excised 120 days after TBI and snap frozen in liquid nitrogen for analysis. Western blot analysis of eNOS, iNOS, PAR-1, β -actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was carried out as previously described (Baker et al. 2008, Strande et al. 2007). Nitrate and nitrite levels in ventricular tissue were measured as described previously (Shi et al. 2005).

Statistical analysis

All values are expressed as the mean $\pm$ standard deviation (SD). For the lipid and liver function, densitometry and echocardiography analysis statistical significance was determined by performing a one-way analysis of variance with Bonferroni's multiple comparison test as the post hoc test. Significance was attributed to those groups that had a P value <0.05 .

Results

Total Body Irradiation

Lipid profile and liver function

From ≥ 20 day following TBI, a sustained increase in serum total cholesterol was seen for the next 40–120 days (Figure 1). LDL cholesterol progressively increased to a peak value of 82 ± 8 mg/dL at 80 days compared with 13 ± 3 mg/dL in unirradiated rats. There was a transient increase in triglyceride levels 40 days after TBI, which then declined to values present in unirradiated rats by 100 days (Figure 1). Serum ALP activity was consistently decreased 40–120 days following TBI. ALT activity was also decreased 100–120 days following TBI, while AST was decreased 40–60 days after TBI. Total bilirubin levels were consistently increased, with albumin and total protein levels decreased 40–120 days following TBI (Figure 2).

Kidney function

Serum urea nitrogen levels were determined 120 days following TBI as 303 ± 31 mg/dL, compared with 22 ± 1 mg/dL in unirradiated control rats.

Blood pressure

After 120 days systolic blood pressure was higher in TBI treated rats (163 ± 7 mmHg) compared with unirradiated rats (120 ± 8 mmHg).

Coronary vessel density

Micro computed tomography showed a reduction in the density of the smaller diameter coronary vessels (<50 μ m diameter) at 120 days after TBI (Figure 3A). The diameter of the epicardial arteries were unaffected by TBI. Three dimensional reconstruction of the coronary network revealed a decrease in the smaller diameter penetrating vessels in 32% of fields examined at random compared with the controls (Figure 3B).

Cardiac histology

The most striking histological lesion seen in hearts 120 days following TBI was the sclerosis of intramural coronary vessels. Affected vessels had partial to complete luminal sclerosis due to concentric laminar thickening of the vessel walls with accumulation of amphophilic matrix material between layers of hyperplastic and vacuolated smooth myocytes. The cardiomyocytes from TBI treated hearts remained normal in appearance (Figure 4A). Trichrome staining revealed peri-arterial fibrosis and irregular collagen deposition around the penetrating coronary

vessels of irradiated hearts. Control hearts had symmetrical penetrating vessels with less collagen and fibrosis (Figure 4B).

Cardiac function

Following TBI, rats had significantly reduced global radial (Figure 5) and circumferential (Figure 5) strain compared to controls as assessed by echocardiography after 120 days. Thus a single exposure to 10 Gy TBI was associated with the development of risk factors for cardiovascular disease, cardiac tissue degeneration and cardiac dysfunction.

Nitric oxide synthase and nitric oxide production

There was a 27% decrease in eNOS and a 29% decrease in iNOS protein after 120 days (Figure 6A). Nitric oxide levels were measured in the heart to determine whether TBI impaired NOS function. Nitrite and nitrate are stable oxidation products of nitric oxide and are used as an index of nitric oxide activity. Nitrite plus nitrate content in hearts from rats exposed to 10 Gy TBI was 20% less than in hearts from control rats after 120 days (Figure 6B).

Circulating fibrinogen levels and expression of PAR-1 in heart

TBI resulted in a 30% increase in expression of PAR-1 protein relative to controls (Figure 7A). Plasma levels of fibrinogen were elevated 21% 120 days after 10 Gy TBI (Figure 7B). Local Thorax Irradiation

Lipid profile and liver function

There were no significant changes observed over the 60–240 days period investigated (Figure 8).

Nitric oxide synthase, fibrinogen and PAR-1 expression

There was no change in expression of eNOS or iNOS protein, nitrite plus nitrate content, fibrinogen or PAR-1 after 240 days (results not shown).

Coronary vessel density and cardiac histology

Histologic examination after 240 days revealed a normal coronary vasculature and cardiomyocyte structure in local thorax-irradiated rats compared with non-irradiated controls (results not shown). There was no structural injury arising from thorax only irradiation.

Cardiac function

Global radial and circumferential strains were not affected at 240 days after local irradiation of the thorax (Figure 9).

Discussion

The results of the present study show that a single TBI exposure with a dose of 10 Gy results in a time-dependent increase in serum total cholesterol, LDL cholesterol, and triglycerides, all of which are biomarkers for the increased risk for cardiovascular disease.

Hypercholesterolemia is associated with morphological injury to the vascular endothelium resulting in stenosis, decreased density of the smaller diameter coronary vessels, and a decrease in ventricular function at 120 days following TBI, manifest functionally as a decline in global radial and circumferential strain. TBI decreased expression of eNOS and iNOS and increased expression of PAR-1 and fibrinogen in the first 120 days after TBI. The dose used in the present study, in young 5 week old rats is considerably less than doses of 15–60 Gy, previously shown by others (Cilliers et al. 1989, Fajardo and Stewart 1970, Lauk 1986, Lauk et al. 1985, Yeung

and Hopewell 1985, Yeung et al. 1989), to cause injury to the heart following local thorax irradiation in adult rats. The study also demonstrated that 10 Gy, local thorax irradiation, does not result in hypercholesterolemia or hypertriglyceridemia, liver enzyme dysfunction or cardiac morphological and ventricular dysfunction even after 240 days.

There is an urgent need to understand the risk of injury to vital organs, such as the heart, following a radiological terrorist attack or nuclear accident, define the mechanisms underlying the injury, and devise treatment strategies. A study published in the *British Medical Journal* (Helfand et al. 2002) estimated that a small 12.5-kiloton nuclear bomb detonated in New York City would cause 50,000 deaths immediately and several hundred thousand cases of radiation-induced sickness. Children would account for a significant portion of this population. An attack on or an accident at a nuclear power plant or waste storage facility poses another radiological threat. An equal concern is the vulnerability of spent fuel pools that can contain 20 to 30 times more radioactive material than a reactor core and are in buildings not nearly as strong as those that house the reactors.

To study the effects of radiation in an animal model closer to potential pediatric patients than in previous studies on the heart, rats were used at the earliest age when they could live independently from the mother. This feature of the experimental design avoids any potential confounding effects arising from nutrition and care that could originate from the mother. WAG/RijCmcr rats are weaned at around 4 weeks of age so that by 5 weeks of age the offspring can exist independently. At 5 weeks of age rats weigh 71 ± 3 g, 41% of the young adult body weight, with well developed hearts, livers and lung, and thus they are likely to be comparable with pre pubescent humans. There is relatively little information about tissue responses to radiation in immature vs. mature rats and what information that does exist is conflicting as it is in humans, however, the consequences of late effects developing in exposed children obviously raised special concerns. To the knowledge of the present authors, no other organ systems in the rat have been evaluated with respect to changes in tolerance between immature and mature animals. The present study is possibly the first to evaluate tolerance to TBI in the immature rat.

The present study shows that risk factors for cardiovascular disease are not increased 60 – 240 days after local thorax irradiation with 10 Gy. In contrast, local irradiation of the heart/thorax using higher doses of 15–60 Gy (Cilliers et al. 1989, Fajardo and Stewart 1970, Lauk 1986, Lauk et al. 1985, Yeung and Hopewell 1985, Yeung et al. 1989) does result in delayed injury to the heart in the same time frame. The present findings suggest that injury to the heart caused by 10 Gy TBI is indirect and is likely caused by dysfunction in other organs resulting in the export of systemic factors that contribute to coronary sclerosis and cardiac ventricular dysfunction. For instance, radiation injury to the kidneys results in proteinuria and hypertension (Moulder et al. 2004). It is well known that proteinuria is associated with hypercholesterolemia as well as low serum albumin and total protein (Wheeler and Bernard 1994). In addition, untreated hypertension is known to contribute to coronary artery sclerosis. Furthermore, serum urea nitrogen is elevated following TBI indicating renal injury is present. Therefore, the detrimental effects on liver lipid metabolism and cardiac function in this study may be a result of radiation-induced renal failure.

The present study describes a persistent increase in total cholesterol and LDL cholesterol with a transient elevation in triglycerides after TBI. Previous studies in rats have described a transient increase in blood lipids in response to 4–8 Gy TBI (Ahlers et al. 1976, Feurgard et al. 1998). This transient increase in plasma cholesterol levels in the rat was associated with increased liver cholesterol synthesis following irradiation (Gould et al. 1959, Shelley 1966). Elevated serum cholesterol levels would be expected in radiation nephropathy because of the associated proteinuria that adversely affects hepatic lipoprotein synthesis resulting in

hypercholesterolemia. Based on AST and ALT levels, as enzyme markers for hepatocyte health, liver function remains fairly stable following irradiation. Most likely, the low serum albumin and total protein is a result of proteinuria, since if it were due to decreased liver synthesis, marked elevations in AST and ALT would be expected, which was not seen.

Hepatic damage, as well as renal damage may be occurring after TBI, as serum albumin levels are decreased after 9.5 Gy TBI (Moulder et al. 1990). Hypoalbuminemia is a symptom of hepatic damage but can also be caused by renal damage. The present study shows that liver function is compromised over the 120 days following TBI, as manifest by increased bilirubin levels and decreased activity of the liver enzymes ALP, ALT and AST (Figure 2). The liver is also damaged by local irradiation (Dawson et al. 2002, Geraci et al. 1992). However, the present study does contrast with the previously observed decline in activity of ALT but not AST in liver homogenates at 85–90 days following local irradiation of rat liver with 10 Gy (Geraci and Mariano 1993). Difference in experimental design, the strain of rat studied and the endpoints used may account for the apparent divergence in outcomes in the present study vs. that of Geraci and Mariano. Albumin and total protein levels are also decreased at 120 days following TBI. However, it is not known if local kidney or liver irradiation increases risk factors for cardiovascular disease. Further studies are needed to determine whether local irradiation of these organs results in increased risk factors for cardiovascular disease, decreased capillary density, and impaired ventricular function to an equivalent level that is present following TBI.

The study also shows that 10 Gy TBI decreases the density of the smaller caliber coronary vessels and confirms previous observations of radiation-induced loss in small vessel density in the heart (Lauk 1987). Nitric oxide plays a central role in vascular biology, so changes in enzyme function will likely have a major impact on endothelial cell and vascular physiology following TBI. It has been shown in the present study that TBI decreases protein levels for constitutive NOS (eNOS), inducible NOS isoforms and nitric oxide generation in the heart. However, the underlying mechanisms are unknown.

TBI results in an increase in plasma fibrinogen levels at 120 days after TBI. Fibrinogen has a plasma half-life of about 4 days. This finding therefore suggests the stimulus for increased production of fibrinogen is still present at 120 days after 10 Gy TBI. TBI also increases expression of PAR-1, the thrombin receptor, in the heart. Activation of PAR-1 may result in proliferation of collagen by increased activation of p42/44 mitogen activated protein kinase (MAPK), which can then cause fibrosis. The role the PAR plays in mediating radiation-induced endothelial dysfunction and the progression to fibrosis in the intestine has been shown (Wang et al. 2007). Taken together with the present findings of increased PAR-1 expression in the heart, further investigation as to the role the PAR plays in radiation-induced injury to the heart are warranted. Radiation also exerts a pro thrombotic effect to increase deposition of von Willebrand factor (Boerma et al. 2004, Verheij et al. 1995). The present findings support the notion that the rat is in a hypercoagulable state 120 days after TBI, most likely as a result of stress response from the liver. Furthermore, clinical and epidemiological studies demonstrate a consistent association between elevated fibrinogen levels and increased risk of atherosclerotic vascular disease. It is proposed that TBI results in an imbalance between procoagulant and anticoagulant function of the vasculature resulting in coronary sclerosis, fibrosis and ventricular dysfunction.

It was also shown in this study that a single 10 Gy dose of TBI results in hypertension by 120 days after irradiation that is associated with hyperlipidemia. It has previously been shown that radiation-induced hypertension over this time course is mediated by the kidney (Cohen and Robbins 2003). Further studies are needed to determine the role of the kidney in radiation-induced abnormal lipid metabolism.

Limitations of the study

The present study was not able to define the sequence of events following TBI that result in cardiac injury. The time course over which cardiac dysfunction is manifest in relation to the appearance of elevated lipid levels, hypertension, and occlusion of the smaller caliber coronary vessels needs to be determined and whether this is associated with renal dysfunction. It has also been shown that TBI but not local thorax irradiation decreases cardiac function as indicated by a decreased strain pattern, but that overall ejection fraction is preserved. Cardiac strain is a very early predictor of heart failure but is still under clinical investigation. Cardiac dysfunction may continue to deteriorate into a loss of ejection fraction in both groups if followed for a longer period of time.

Conclusion

10 Gy TBI worsened the blood lipid profile in a rat model, resulted in coronary microvasculature injury, altered endothelial physiology and myocardial mechanics. Injury to the heart following TBI may be indirect with non-thoracic organs contributing to coronary sclerosis and ventricular dysfunction. The mechanisms underlying TBI-induced injury to the heart need to be understood and treatments developed to decrease the risk of developing cardiovascular disease after accidental or intentional exposure.

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Figure 1.
Time-related changes in total cholesterol, LDL cholesterol and triglycerides following TBI.
Data shown as means $\pm$ SD, n=6/group. * $p < 0.05$, 10 Gy vs controls.

Figure 2.

Time-related changes in liver dysfunction assessed by measurements of three liver enzymes ALP, ALT and AST plus serum bilirubin, albumin and total protein. Data shown as means $\pm$ SD, n=6/group. * p<0.05, 10 Gy vs controls.

Figure 3.

Coronary vessel changes. A. Micro computerized tomography images of isolated rat heart with contrast agent perfused through the coronary arteries. Representative image of a heart obtained 120 days after exposure to 10 Gy compared with an age matched control. The limit for visualization of coronary vessel diameter is 4 μm . B. Three dimensional micro computed tomography reconstruction of coronary network showing density of coronary vessels at 120 days following 10 Gy TBI compared with an age matched control. TBI resulted in a decrease in the density of the smaller diameter coronary vessels ($<50 \mu\text{m}$). The density of large diameter coronary vessels was unchanged following 10 Gy TBI.

Figure 4.

Morphological changes. A. Heart sections stained with H&E show vessel lumen completely blocked (➡) as a result of myointimal proliferation 120 days after 10 Gy TBI. Three hearts were studied in each group. The lumen of a comparable vessel in an age matched heart is patent and contains red blood cells (⇨). B. Heart section stained with Trichrome showing increased peri-arterial fibrosis in small caliber coronary vessel 120 days after 10 Gy TBI compared with a comparable vessel in an age matched control. Three hearts were studied in each group. Fibrosis appears as blue using trichrome staining.

Figure 5. Changes in ventricular function at 120 days after 10 Gy TBI compared with age matched controls. Data shown as means $\pm$ SD, n=6/group. * p<0.05, 10 Gy vs age matched control.

Figure 6.

Variation in iNOS and eNOS protein content in heart 120 days following exposure to 10 Gy TBI compared with age matched controls. A. Western analysis of iNOS and eNOS expression in heart homogenates B. Changes in nitrite plus nitrate content in heart 120 days after TBI. Data shown as means $\pm$ SD, n=3/group. * = $p < 0.05$, 10 Gy vs aged matched control.

Figure 7.

Changes in coagulant function following TBI. A. Changes in the expression of PAR-1 in heart homogenates 120 days after exposure to 10 Gy TBI compared with controls. Data shown as means $\pm$ SD, $n=3$ /group. B. Changes in fibrinogen levels in the plasma 120 days after exposure to 10 Gy TBI compared to aged matched controls. Data shown as means $\pm$ SD, $n=6$ /group. * $p<0.05$, 10 Gy vs age matched control.

Figure 8.

Time related changes in blood lipid and liver function after local thorax irradiation. Data shown as means $\pm$ SD, n=6/group.

Figure 9. Ventricular function 240 days after local thorax irradiation with 10 Gy compared to age matched controls. Data shown as means $\pm$ SD, n=3/group.