L-Arginine Mitigates Radiation-Induced Early Changes in Cardiac Dysfunction: The Role of Inflammatory Pathways

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Aims of the Study

Our earlier studies demonstrated the ability of L-arginine (L-Arg) to reverse radiation-induced immune dysfunction. The aim of the present study was to investigate cardiac dysfunction up to 24 h after 2 Gy of total-body irradiation (TBI) and its mitigation by L-Arg. The current studies also explored the association of radiation-induced inflammation and electrocardiographic (ECG) abnormalities, TBI-induced cardiac iNOS and kinin B1 R, changes in the ECG profile like bradycardia, increased RR interval, ST elevation and increased QRS duration at 4 h and 24 h after TBI. TBI with 2 Gy induced inflammatory responses in spleen and cardiac tissue. L-Arg administered 2 h after TBI (TBI + L-Arg) mitigated the entire inflammatory response and ECG profile toward normalcy. L-Arg administered just before TBI (L-Arg + TBI) could not reverse the above-mentioned changes. Radiation-induced inflammatory responses at +4 h and +24 h after TBI in spleen and cardiac tissue correlated with the changes in ECG profile at the corresponding time. The results suggest the ability of L-Arg administered at the correct therapeutic window to mitigate radiation-induced cardiac dysfunction at 4 and 24 h after TBI.

INTRODUCTION

Exposures to doses of radiation of 1–10 Gy may occur during the course of radiation therapy or as the result of radiation accidents or nuclear/radiological terrorism (1). An inflammatory response and immune dysfunction is a classical feature of radiation exposure and appears to be a key event in the development of radiation injury (2). Radiation-induced heart disease has been established as indicating the clinical and pathological conditions resulting from injury to the heart during therapeutic irradiation of adjacent neoplasm (3–5). Inflammation caused by proinflammatory cytokines such as IL-1β, TNF-α and IL-6 have been implicated in critical injuries caused by acute radiation injury, heatstroke, sepsis and clinical pathological conditions such as SIRS, inflammatory myocarditis, cardiac allograft rejection and heart failure (2, 6–9). Nitric oxide (NO) produced by iNOS has also been suggested to play a role in the pro-inflammatory cytokine-induced cardiac contractile failure.

Early inflammatory changes in the heart after total-body irradiation (TBI) have not been studied so far. In addition, no one has reported the early cardiac changes induced by radiation and its association with the inflammatory response. Our initial work had indicated that TBI of mice caused changes in the ECG pattern (10). Considering the above, studies were designed to see the effect of a dose of 2 Gy on heart electrical activity at 4 and 24 h after TBI and its association with cardiac and splenic inflammatory markers in the irradiated host. The earlier studies had suggested the importance of the time of administration of L-Arg and the L-Arg pathway in mitigating the radiation-induced host immune dysfunction and inflammation caused by heatstroke, sepsis (2, 6–8). Based on the above, experiments were designed to determine the ability of L-Arg to mitigate the inflammatory process and the early cardiac dysfunction when administered at the correct therapeutic window. In this study, we used a murine model, because it is the best characterized animal model for initial assessment of therapeutic agents to mitigate radiation injury (11).

MATERIALS AND METHODS

Animals

Swiss/Bh inbred male mice weighing 25–27 g and 7–8 weeks of age were used for the studies; the animal care was as described previously (2). All the experiments were carried out in accordance with the ethical guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

Total-Body Irradiation and L-Arg Administration

Mice were divided into five groups consisting of four mice each. Sham-irradiated mice were handled similarly but were not irradiated...
TABLE 1
Primer Sequences for Amplification of cDNA during PCR with their Respective Annealing Temperatures

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>Forward 5'-CATCCTAAAAATTGGGACAGC-3'</td>
<td>47°C</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GGACTGTATGTTGGCTGGA-3'</td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>Forward 5'-AAGGATGTTGGGAGAGGAGA-3'</td>
<td>50°C</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GGGAGTTGCTGAGGCATGGA-3'</td>
<td></td>
</tr>
<tr>
<td>Kinin B1 R</td>
<td>Forward 5'-CAGGTCTTGTGGACTCTCTCAC-3'</td>
<td>54°C</td>
</tr>
<tr>
<td>IL-6</td>
<td>Forward 5'-GGGAGTCAAGGAGGAGGCTAAGG-3'</td>
<td>57°C</td>
</tr>
<tr>
<td>IL-4</td>
<td>Forward 5'-ATCCGCATTGGAGAGGAGGCA-3'</td>
<td>53°C</td>
</tr>
<tr>
<td>IL-10</td>
<td>Reverse 5'-CTTATCGGATGATACAGGCA-3'</td>
<td>56°C</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Forward 5'-TGGACCGCACAACAGCCCACTATGAGAAA-3'</td>
<td>59°C</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Reverse 5'-CAGGACAGGTTATAGATTCTTTCCTTT-3'</td>
<td>53°C</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Reverse 5'-CTTATCAGGATGATACAGGCA-3'</td>
<td>55°C</td>
</tr>
<tr>
<td>GATA-3</td>
<td>Forward 5'-GAGGCTGAACTATGGGAGAGGCA-3'</td>
<td>50°C</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GAGGCTGAACTATGGGAGAGGCA-3'</td>
<td></td>
</tr>
<tr>
<td>T-bet</td>
<td>Reverse 5'-GCCAGGGAAGGCAAGCCTCATAG-3'</td>
<td>52°C</td>
</tr>
</tbody>
</table>

(Sham group). Mice were exposed to 2 Gy 60Co γ rays (Atomic Energy Canada, Model 220) at a dose rate of 10.79 Gy/s (TBI group). L-Arg (Sigma Chemical Co., St. Louis, MO) in normal pyrogen-free physiological saline (120 mg/kg) was administered intraperitoneally (i.p.). The i-Arg + TBI group received the i-Arg 10 min before TBI and those in the TBI + i-Arg group received i-Arg 2 h after TBI. A group of normal mice received only i-Arg at 0 h (i-Arg group). After treatment, mice were given sterile food and acidified water ad libitum. Care was taken to ensure that the bedding of the cages was changed twice a day. The time of TBI was considered as 0 h, and various parameters were monitored with reference to this time.

Semi-quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Since RT-PCR has been used to monitor the possible biomarkers for ionizing radiation exposure in human blood lymphocytes, we used this method to monitor the upregulation of inflammatory molecules in the irradiated host (2). Expression of various cytokines, iNOS, kinin B1 R and transcription factor T-bet and GATA-3 was monitored by one-step RT-PCR in spleen and heart as reported earlier (2). For RNA preparation, splenic and cardiac tissue were pooled from four mice per group per experiment. The RNA was reverse-transcribed by one-step RT-PCR by using the Masterscript™ RT-PCR System (5 Prime GmbH, Germany) as described earlier (2). The program used for one-step RT-PCR was as reported earlier with the exception of the annealing temperatures for 45 s for various primers (Table 1). The sequences of oligonucleotide primers of β-actin, iNOS, kinin B1 R, various cytokines, T-bet and GATA-3 are presented in Table 1. Semi-quantitative RT-PCR was performed using β-actin as an internal control to normalize gene expression for the PCR templates. The absence of PCR product signal from genomic DNA contamination was confirmed by performing PCR with representative RNA samples without reverse transcription amplification of mRNA. Equal amounts of each PCR reaction product (10 μl) were run on 2% agarose gels containing ethidium bromide in Tris borate EDTA buffer at 60 V. The intensity of the bands in the gels was visualized under a UV lamp, and relative intensities were quantified using GeneSnap Software (Syngen).

Animal Preparation for ECG

ECGs were performed with a Bio Amp device PowerLab System (PowerLab 2/20; ADInstruments, Australia), which recorded bipolar lead I. For the ECG recording, all animals were anesthetized by i.p. injection of urethane (1 g/kg body wt), and the recordings were taken in a supine position. All the recording were performed between 10:00 a.m. and 2:00 p.m. to exclude the influence of diurnal variations or to minimize circadian variations in a constant environment. Once the corneal and withdrawal reflexes were absent in the mice, we inserted MLA1204 needle electrodes subcutaneously into the right forelimb and into each hind limb. The needle electrodes and pulse transducer (ADInstruments) were connected to a PowerLab/4SP (ADInstruments) through an ML136 Animal Bio Amp. Mice were allowed a 45-min stabilization period before obtaining baseline ECG and HR recordings. The procedure was done to avoid the inclusion of any artifact due to anesthesia. Single-channel (lead II) ECG was recorded for 20 min. For ECG recordings, four mice per group per experiment were used.

Data Acquisition and Analysis

The ECG signal was amplified using a PowerLab4SP system and was digitized at a sampling rate of 1 kHz. All data were acquired on a computer for further analysis using Chart 5 for Windows software. Wave durations (in milliseconds) were calculated automatically by the software after placement of the cursors. Measurements were average values determined from 20-min consecutive ECG records. Records were filtered (1 to 100 Hz) through a band-pass filter to minimize environmental signal disturbances. ECG signal output was recorded with Chart 5 for Windows. Data were analyzed using raw data for the ECG signals acquired at a sampling rate of 1 K/s, lower pass 0.3 Hz and upper pass 1 kHz. RR interval, QRS duration and the peak height of T wave were analyzed using Chart 5 Pro (Version 5.5 for Windows). The ECG analysis included the following measurements:
FIG. 1. Splenic expression of (panel A) TNF-α, (panel B) T-bet, (panel C) TGF-β and (panel D) GATA-3 mRNA after TBI as determined by RT-PCR as described in the Materials and Methods. Gene expression data were normalized to β-actin expression. Band intensity is shown as means ± SEM of data from three independent animal experiments (three biological replicates) with similar results. In each experiment, spleens from four mice were pooled for RNA isolation per group. *P < 0.05 compared to Sham group. †P < 0.05 compared to TBI group, **P < 0.05 compared to L-Arg + TBI group. Panel E: The splenic expression of GATA-3, TGF-β, TNF-α, T-bet and β-actin mRNA 4 and 24 h after TBI. Lanes 1 to 5 are Sham, TBI, L-Arg + TBI, TBI + L-Arg and L-Arg, respectively. When there was not a basal level of expression, the bands are not shown. The PCR band shown is representative of the three independent animal experiments with similar results.
FIG. 2. Cardiac expression of (panel A) TNF-α, (panel B) IL-1β, (panel C) IL-6, (panel D) T-bet, (panel E) iNOS and (panel F) kinin B1 R mRNA after TBI by RT-PCR as described in the Materials and Methods. Gene expression data were normalized to β-actin expression. Band intensity is shown as means ± SEM of data from three independent animal experiments (three biological replicates) with similar results. In each experiment, hearts from four mice were pooled for RNA isolation per group. *P < 0.05 compared to Sham group, $P < 0.05$ compared to TBI group, **P < 0.05 compared to L-Arg+TBI groups. Panel G: Cardiac expression of kinin B1 R, iNOS, IL-6, IL-1β, TNF-α, T-bet and β-actin mRNA at 4 and 24 h. Lanes 1 to 5 are Sham, TBI, TBI+L-Arg, TBI+L-Arg and L-Arg, respectively. When there was not a basal level of expression, the bands are not shown. The PCR band shown is representative of the three independent animal experiments with similar results.
heart rate in beats per min, RR interval in ms, ST amplitude in μV and QRS duration in ms. The software used a derivative-based QRS detection algorithm to calculate the heart rate by detecting the peaks of the R waves automatically.

Preparation for Heart Histology

Light microscopy analysis of the left ventricle of heart tissue was performed on tissue slices fixed in neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. Stained tissue sections were examined microscopically at 1000× magnification and recorded by a Carl-Zeiss microscope attached to a Nikon Camera.

Assay for Serum Markers for Cardiac Injury

Activity of creatine kinase (CK) and its isoenzyme CK-MB was assayed in serum using a commercially available colorimetric kit (Agappe Diagnostics, Mumbai) following the manufacturer’s protocol. The serum LDH levels were assayed using commercially available colorimetric kits (Ecoline R; Merck Limited, Mumbai) following the manufacturer’s protocol. Briefly, 50 μl of serum was added to 1000 μl of reaction solution (4:1). The resultant reaction mixture was incubated at 37 °C for 5 min and the absorbance of the solution was read at 340 nm.

Immunohistochemical Staining for T Cells and Macrophages in Cardiac Tissue

For immunostaining, hearts from treated animals were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained for CD3 T cells or CD14 macrophages as described previously (13). Briefly, sections were deparaffinized, rehydrated using xylene and graded series of ethanol and distilled water, permeabilized and stained with PE-labeled mouse anti-CD3 (BD Biosciences) for the presence of T cells or with FITC-labeled mouse anti-CD14 (BD Biosciences) for the presence of macrophages. Section were then analyzed using an LSM510 confocal microscope (Carl Zeiss, Jena GmbH, Germany) with a krypton-argon and He-Ne laser coupled to an Orthoplan Zeiss photomicroscope.

Statistical Analyses

Values are expressed as means ± SEM unless otherwise stated. Data from all the experiments were analyzed using one-way ANOVA followed by post-hoc analyses using the Scheffe test. P < 0.05 was considered to be statistically significant.

RESULTS

L-Arg Mitigates Radiation-Induced Splenic Inflammatory Cytokine Response

Mouse spleens in the TBI and L-Arg + TBI groups had significantly increased expression of TNF-α and T-bet compared to the Sham group at 4 and 24 h (Fig. 1A, B, E). In contrast, mouse spleens in the TBI + L-Arg group had significantly reduced expression of both TNF-α and T-bet compared to the TBI and L-Arg + TBI groups. Mice in the TBI + L-Arg group had significantly increased expression of TGF-β and GATA-3 compared to the TBI and L-Arg + TBI groups. Mice in the TBI and L-Arg + TBI groups at 24 h (Fig. 1C, D, E). Mice in the TBI + L-Arg group had significantly increased expression of T-bet, TGF-β and GATA-3 in their spleens. Expression of these genes at the mRNA level are shown in Fig. 1E. The expression of IL-4, IL-10 and IL-6 was found to be absent in all five groups at 4 and 24 h (data not shown).

L-Arg Mitigates Radiation-Induced Heart Inflammatory Cytokine Response

Mouse hearts in the TBI and L-Arg + TBI groups had significantly increased expression of TNF-α, IL-6 and T-bet compared to the Sham group at 4 and 24 h (Fig. 2A, C, D, G). Mice in the TBI + L-Arg group had significantly decreased expression of TNF-α, IL-6 and T-bet compared to the TBI and L-Arg + TBI groups. Expression of IL-1β in mouse hearts was found to be elevated at 4 h only in the TBI and L-Arg + TBI groups compared to the Sham group and was significantly reduced in the TBI + L-Arg group compared to the TBI and L-Arg + TBI groups (Fig. 2B, G). IL-4, IL-10, GATA-3 and TGF-β were not
expressed in any of the groups at 4 and 24 h (data not shown).

L-Arg Mitigates Radiation-Induced Cardiac iNOS and Kinin B1 R Expression

Mouse hearts in the TBI and L-Arg+TBI groups had significantly increased expression of iNOS and kinin B1 R compared to the Sham group (Fig. 2E, F, G). Mice in the TBI+L-Arg group had significantly decreased expression of iNOS and kinin B1 R compared to the TBI and L-Arg+TBI groups in their hearts. The expression of these genes at the mRNA level is shown in Fig. 2G.

L-Arg Mitigates Radiation-Induced Bradycardia

The anesthetized mice were assessed at 4 and 24 h after TBI for ECG patterns (Fig. 3). The mice exposed to radiation showed significant bradycardia and an increased RR interval (Fig. 4A, B). Bradycardia in mice in the L-Arg+TBI group increased significantly compared to mice from either the Sham and TBI groups. Mice in the TBI+L-Arg group had significantly reduced bradycardia and RR interval compared to the TBI and L-Arg+TBI groups.

L-Arg Mitigates Radiation-Induced ST Elevation

The mice exposed to radiation showed ST elevation (Fig. 4C). The ST elevation in L-Arg+TBI mice was significantly higher compared to mice in the Sham and TBI groups. Mice in the TBI+L-Arg group had significantly decreased ST amplitude compared to the TBI and L-Arg+TBI groups.

FIG. 3. Continued

L-Arg Mitigates Radiation-Induced Increase in QRS Duration

The TBI group showed increased QRS duration (Fig. 4D). The QRS duration in L-Arg+TBI mice was significantly higher compared to the Sham group. Mice in the TBI+L-Arg group had a significantly reduced QRS duration compared to either TBI and L-Arg+TBI groups.

Heart Histology, Immunostaining and Serum Markers for Cardiac Injury

The heart histology did not show any significant myocardial structure disorder or necrosis (Fig. 5). To assess the presence of inflammatory cells in the cardiac tissue of treated animals, we used double immunofluorescence staining and confocal analysis. It was found that compared to the control (Sham) group, the irradiated groups did not show fluorescence of either CD3 (red) or CD14 (green), demonstrating the absence of T cells or macrophages in the cardiac tissue at both 4 and 24 h (Supplementary Figs. 1 and 2, respectively; http://dx.doi.org/10.1667/RR2523.1.S1). The serum levels of LDH, CK and CK-MB were studied as markers for cardiac injury and were not significantly different any of the four groups compared to the Sham group (data not shown).

DISCUSSION

The aim of these studies was to look at the changes in cardiac electrical activity and the gene expression of
inflammatory mediators 4 and 24 h after TBI. The results from the current experiments indicated that mice in the TBI and L-Arg+z-TBI groups had marked bradycardia, increased RR interval, ST elevation, increased QRS duration and increased expression of inflammatory markers in cardiac and splenic tissue. L-Arg administered 2 h after TBI (TBI+z-L-Arg group) shifted the entire cardiac and inflammatory parameters toward normal. These studies correlate with our earlier studies that suggested the ability of L-Arg to reverse the radiation-induced immune dysfunction when administered after TBI (2). Our results also suggested that there was no structural damage in the heart tissue of irradiated mice, as indicated by histology, immunohistochemical staining for T cells and macrophages, and markers for cardiac injury in serum. Alterations in the ECG profile suggest that there are functional alterations in the heart tissue. Since the ECG evaluates the electrical activity of the heart our results suggest that TBI causes significant changes in the electrical activity of the heart. These new data helped us in designing an anti-inflammatory approach to mitigate radiation-induced cardiac dysfunction, based on the earlier studies, which demonstrated the ability of L-Arg administered postirradiation in reversing radiation-induced inflammation and immune dysfunction (2). Supplemental dietary arginine given after abdominal irradiation accelerated intestinal mucosal regeneration and enhanced bacterial clearance after radiation enteritis in rats (14). However, when L-Arg was fed to rats before exposing the abdomen to radiation, significantly increased damage to various segments of intestine was seen compared to rats on other feeding regimens (15). These observations are line with our findings and suggest the importance of the correct time window for L-Arg administration (2). Our studies indicated that L-Arg administration prior to radiation

FIG. 4. Means ± SEM of (panel A) heart rate (beats/min), (panel B) RR interval (ms), (panel C) ST height (μV), and (panel D) QRS duration (ms) in mice 24 h after TBI. Data are from one of the four independent animal experiments with similar results. In each experiment, data from four mice per group were assessed. Analysis was done using Chart 5 Pro software for 20 min of data acquisition after 60 min of normalization. *P < 0.05 compared to Sham group, **P < 0.05 compared to TBI group, ***P < 0.05 compared to L-Arg+z-TBI group.
exposure accelerated the production iNOS, which contributes to increased NO production and worsens the response of radiation injury. l-Arg administered after radiation exposure significantly reduced the levels of iNOS with a concomitant increase in the levels of arginase, indicating the importance of the time of administration of l-Arg in shifting the arginine pathway away from production of excess NO. The studies offer a mechanistic explanation by demonstrating the importance of the time of l-Arg administration in modulating the l-Arg pathway away from excess production of NO in the therapy of ARS. In the current studies, l-Arg administered just before TBI (l-Arg + TBI group) caused further ECG changes compared to TBI alone, again supporting the earlier work on the importance of the time of administration of l-Arg and the role of arginine pathways in altering the course of radiation injury (2).

Results from cancer patients treated with radiation indicate that radiation-induced heart disease is a long-term complication of radiation exposure (3–5). Manifestations of radiation-induced heart disease include accelerated atherosclerosis, pericardial and myocardial fibrosis, conduction abnormalities and injury to cardiac valves (16). Release of proinflammatory cytokines like TNF-α, IL-1β from macrophages and monocytes aggravate the radiation-induced inflammatory cascade. Activation of these cytokine cascades play a role in the development of late radiation effects (17). Our studies demonstrate that changes occurred in ECG and gene expression of inflammatory mediators within 24 h after TBI, which has not been reported before. Hence halting
the inflammatory cascade at an early stage after irradiation would be an approach to treat the development of radiation-induced heart disease. Therefore, treatment of mice with L-Arg after TBI, which prevents the expression of early inflammatory mediators induced by radiation, could contribute to preventing the development of long-term complications of radiation.

Radiation-induced inflammatory responses and their potential side effects on the host have been demonstrated (2, 18). The differentiation of naïve T-helper (Th) cells to Th1 or Th2 cells is regulated by the transcription factors T-bet and GATA-3, respectively. T-bet/GATA-3 ratio has been used as a measure of the Th1/Th2 cytokine profile in mixed cell populations (19). The T-bet/GATA-3 ratio in the spleen at 24 after TBI increased to 5.1 in the TBI group compared to the Sham group, increased further to 6.25 in the T-Arg + TBI group, and decreased to 0.25 in the TBI + l-Arg group. The decreased T-bet/GATA-3 ratio and Th1/Th2 ratio in the spleens of mice in the TBI + l-Arg group suggest the reversal of the inflammation. Increased TNF-α and T-bet in the spleen and heart in the l-Arg + TBI group correlated with depressed cardiac function. These decreased inflammatory markers in the spleen and cardiac tissue from the TBI + l-Arg group correlated with the reversal of the cardiac dysfunction. The cascade of cytokines and the corresponding transcription factors produced immediately after irradiation contributed to the course of radiation injury. Our studies suggest that therapy to treat the cardiac dysfunction should aim to lower the levels of TNF-α and T-bet. These results suggest that for immediate reversal of the injury, the Th1/Th2 ratio should be in favor of Th2 cytokines. Our findings are in agreement with our previous studies of acute injuries like acute radiation syndrome, heatstroke and sepsis, which suggest the need to shift the Th1 pathway away from excess NO production (2, 6–8) to initiate host recovery from the injury. The work demonstrated that exogenous administration of l-Arg at 2 h after TBI induces host homeostasis, which may be critical in establishing conditions suitable for an organism to respond to radiation injury. The ability of l-Arg to upregulate both the transcription factors T-bet and GATA-3 and the cytokine TGF-β in the spleen (Fig. 1) correlates with our earlier studies of the increased splenic proliferation with the administration of l-Arg (2). In this way, immunomodulator like l-Arg administered at the right dose and time may mitigate the radiation-induced immune dysfunction.

Kinin B1 R is one of the most profoundly regulated G-protein-coupled receptors and is not expressed in normal physiology but is induced during inflammation (6, 20, 21). Normal functioning of kinin B1 R is important for mammalian heart physiology (21). Kinin B1 R activation plays a significant role in the progression of inflammatory cardiovascular disease, and kinin B1 R-antagonists has beneficial therapeutic effects in halting the progression of inflammatory cardiovascular disease (20, 23). Ours is the first report of the role of kinin B1 R in radiation-induced cardiac dysfunction. Since l-Arg has been shown to downregulate the expression of kinin B1 R in heatstroke (6), we tested the ability of l-Arg to suppress the radiation-induced expression of cardiac B1 kinin R. The results convincingly demonstrate the ability of l-Arg to suppress radiation-induced expression of kinin B1 R and bring the cardiac function of the irradiated host toward normalcy.

Excessive production of NO induced by radiation is involved in radiation injury (2, 24). NO plays a central role in cardiovascular regulation (21). Excessive NO production by iNOS contributes to profound cellular disturbances leading to heart failure (25, 26). NO produced by iNOS is syth班组hibitory and is known to induce hypotension and bradycardia (27). The increased iNOS gene expression in cardiac tissue and bradycardia in mice in the TBI group supports this observation. In the current work, mice given l-Arg before TBI had pronounced bradycardia with a concomitant increase in iNOS gene expression. The decrease in iNOS gene expression in the group of mice given l-Arg at 2 h after TBI correlates with the reversal of bradycardia. Induction of Th1 cytokines TNF-α and If-1β is associated with an increase in iNOS activity (28, 29). Enhanced NO generation from iNOS was thought to be responsible for marked cardiac dysfunction as seen in most inflammatory heart diseases (30, 31). Increased cardiac iNOS contributes to depressed myocardial contractility and beta-adrenergic responsiveness in heart failure rats (30). Selective iNOS blockade improved the cardiac function. In the current work, l-Arg administered at 2 h after TBI significantly reduced cardiac iNOS, kinin B1 R and inflammatory cytokines. These results suggest that regulation of pro-inflammatory molecules and control of NO release by myocardial iNOS by the administration of l-Arg at the correct therapeutic window may be a good therapeutic strategy for the treatment of cardiac diseases.

In the present study, radiation-induced expression of inflammatory molecules, kinin B1 R and iNOS in the heart was accompanied by altered electrical activity of the heart. Earlier studies in rats did not find statistically significant differences in the ECGs monitored 24 h after 15 Gy irradiation of the heart (32). However, in our studies we observed significant ECG changes at 4 h after exposure to 2 Gy that persisted until 24 h after TBI. This suggests that alterations in ECGs caused by radiation are due to complex interactions of central nervous system, the immune system and other systemic responses. In general, ST segment elevation reflects myocardial injury (33). There was no significant increase in the levels of CK, CK-MB and LDH in serum (data not shown) and structural alterations in the heart histology. The results suggest that immediate alteration of the electric property of the heart and not the functional expression.
of the damage is responsible for the changed ECG profile. Changes observed in QRS duration indicate either nonspecific intraventricular conduction delays or ectopic rhythms originating in the ventricles due to the disturbances in pacemaker rhythm, which is a clear indication of altered cardiac excitability (34, 35).

Our current studies along with earlier studies demonstrate that L-Arg when administered at the correct time can reverse both cardiac and immune dysfunction (2). However, it is not always necessary that gene expression reflect the changes at protein or enzyme levels. Further confirmation of these data is required at the protein or enzyme level. These results could form the basis of detailed studies in mice regarding the long-term effects of radiation on cardiac function, inflammation of the host and its modulation by L-Arg. Exogenous administration of L-Arg after TBI induces host homeostasis, which may be critical in establishing conditions in which an organism can respond to radiation injury. The pathophysiological mechanisms of these associations remain to be elucidated. This may have implications for military and civilian triage where L-Arg can be remain to be elucidated. This may have implications for military and civilian triage where L-Arg can be

radiation exposure and to identify potential biological diagnostic and prognostic indicator of radiation injury.

Because L-Arg is a well-characterized least toxic amino acid whose pharmacodynamics is well characterized, it can easily enter clinical trials.

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