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Radiation-induced afferent arteriolar endothelial-dependent dysfunction involves decreased epoxygenase metabolites

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Imig JD, Hye Khan MA, Sharma A, Fish BL, Mandel NS, Cohen EP. Radiation-induced afferent arteriolar endothelial-dependent dysfunction involves decreased epoxygenase metabolites. Am J Physiol Heart Circ Physiol 310: H1695–H1701, 2016. First published April 22, 2016; doi:10.1152/ajpheart.00023.2016.—Chronic kidney disease is a known complication of hematopoietic stem cell transplant (HSCT) and can be caused by irradiation at the time of the HSCT. In our rat model there is a 6- to 8-wk latent period after irradiation that leads to the development of proteinuria, azotemia, and hypertension. The current study tested the hypothesis that decreased endothelial-derived factors contribute to impaired afferent arteriolar function in rats exposed to total body irradiation (TBI). WAG/RijCmcr rats underwent 11 Gy TBI, and afferent arteriolar responses to acetylcholine were determined at 1, 3, and 6 wk. Blood pressure and blood urea nitrogen were not different between control and irradiated rats. Afferent arteriolar diameters were not altered in irradiated rats. Impaired endothelial-dependent responses to acetylcholine were evident at 3 and 6 wk following TBI. Nitric oxide synthase (NOS), cyclooxygenase (COX), and epoxygenase (EPOX) contribution to acetylcholine dilator responses were evaluated. NOS inhibition with Nω-nitro-l-arginine methyl ester (l-NAME) reduced acetylcholine responses by 50% in controls and 90% in 3-wk TBI rats. COX inhibition with indomethacin did not significantly alter the acetylcholine response in the presence or absence of l-NAME. EPOX inhibition with 4-(methylsulfonyl)phenyl-2-propargylamine significantly decreased acetylcholine responses (35%) in controls but did not significantly alter acetylcholine responses (4%) in TBI rats. Biochemical analysis revealed decreased urinary EPOX metabolites but no change in COX, NOS, or reactive oxygen species at 3 wk TBI. Taken together, these results indicate that afferent arteriolar endothelial dysfunction involves a decrease in EPOX metabolites that preceeds the development of proteinuria, azotemia, and hypertension in irradiated rats.

NEW & NOTEWORTHY

Renal microvascular endothelial dysfunction following total body irradiation is progressive and occurs before azotemia, proteinuria, and hypertension. Therapeutic approaches to increase renal endothelial-derived epoxyenase metabolites could be beneficial for treating radiation-induced nephropathy.

HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) is used worldwide for the treatment of cancer, and over 50,000 people per year now undergo HSCT (4, 8, 32, 37). Long-term survival after HSCT is now near 50%. Thus, more people will have late effects after HSCT, which include chronic kidney disease (CKD) in 10–20% of survivors or 5,000 new cases of CKD per year (4, 8, 32, 37). CKD in HSCT patients has been clearly linked to irradiation at the time of HSCT (4, 8). Radiation nephropathy also occurs after radionuclide therapy for cancer and could occur with partial or whole body irradiation from accidental exposure or radionuclear attack (8, 9, 28). Early renal endothelial and vascular injury could contribute to radiation nephropathy (1, 22, 23, 34, 36). Micropuncture studies in rats have documented an increase in renal vascular resistance starting at 45 min and persisting at day 60 following a 15-Gy single fraction renal irradiation (41). Likewise, Robbins and Hopewell showed persistent reduction in effective renal plasma flow in a porcine model that used 10.7 Gy single-fraction irradiation (33). Ultrastructural studies suggest a contribution for endothelial injury in radiation nephropathy (34). Impaired endothelium-dependent vasodilatation has been reported as early as 8 days after irradiation; however, these studies were done using a high dose of irradiation that is above the clinically relevant range (23). Therefore, there is a significant need to gain a better understanding of renal microvascular and endothelial function following irradiation.

Renal microvascular and afferent arteriolar endothelial dysfunction have been demonstrated in several cardiovascular and renal diseases (16, 18). Afferent arteriolar endothelial-dependent dilator responses involve nitric oxide synthase (NOS), cyclooxygenase (COX), and cytochrome P-450 epoxygenase (EPOX) metabolites (16, 19). Previous studies have demonstrated that endothelial-derived nitric oxide and epoxyeicosatrienoic acids (EETs) are responsible for a large part of the afferent arteriolar dilator responses to bradykinin and acetylcholine (19, 42, 45). There is also evidence that decreased nitric oxide and increased reactive oxygen species contribute to vascular dysfunction following irradiation in the rat mesenteric microvessels and human cervical arteries (3, 12, 29, 38, 39, 46). However, intrarenal afferent arteriolar changes in endothelial-dependent dilatation and alterations in endothelial-derived metabolites following irradiation are not known.

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Radiation nephropathy presents clinically as proteinuria, azotemia, and hypertension (4, 9). We have replicated these features in our models of experimental radiation nephropathy, which we have used extensively to evaluate the progression of the renal failure that occurs after single or multiple irradiation fractions. There is a 6- to 8-wk latent period after irradiation that is followed by proteinuria, azotemia, and hypertension in rats (4, 6). This supports the notion that early vascular injury is a critical component to radiation nephropathy.

Although irradiation results in nephropathy and hypertension, there have been only a limited number of experimental studies evaluating renal vascular endothelial function following radiation. Accordingly, this experimental study was undertaken to determine the time course of alterations in afferent arteriolar endothelial-dependent dilator responses following irradiation and before the development of proteinuria, azotemia, and hypertension. Additional experiments were conducted to evaluate the involvement of endothelial-derived factors to impaired afferent arteriolar dilator responses in irradiated rats.

MATERIALS AND METHODS

Animal model of radiation nephropathy. Studies were performed in syngeneic 7- to 8-wk-old male WAG/RijCmcr rats that were bred and housed in a moderate security barrier. Rats were maintained in the Biomedical Resource Center at the Medical College of Wisconsin. The Institutional Animal Care and Use Committee of the Medical College of Wisconsin approved animal protocols. Total body irradiation (TBI) was performed using a X-RAD 320 orthovoltage unit (Precision X-Ray, North Branford, CT) as described earlier (27). Rats were immobilized in a Plexiglas jig that restricted their movement. TBI (11 Gy) was delivered posteriorly to anteriorly. The half-value layer of the beam used for irradiation was 1.4 mm of Cu, and it was given at a dose rate of 1.75 Gy/min. Bone marrow was partially spared by shielding a hind leg with a 0.25-in.-thick lead block. Systolic blood pressure was continuously monitored by a tail-cuff method (Kent Scientific, Torrington, CT), and urine was collected over a 24-h period on the 3rd day after irradiation. Six weeks after irradiation, rats were anesthetized for blood sample collection followed by death and tissue collection. The levels of blood urea nitrogen (BUN) were measured spectrophotometrically using commercial kits (BioAssay Systems, Hayward, CA).

Microvascular preparation. Rats were anesthetized with pentobarbital sodium (40 mg/kg body wt ip), and a midline abdominal incision was made. The renal artery of the right kidney was cannulated via the superior mesenteric artery, and the kidney was immediately perfused with a Tyrode solution containing albumin and a mixture of L-amino acids (20). The kidney was excised and maintained in an organ chamber throughout the isolation and dissection procedure. The juxtamedullary nephron region was isolated so that the vasculature could be visualized directly. Large branches of the renal artery were ligated with fine suture, and the arterial supply of the exposed microvasculature was isolated. After the dissection was completed, the Tyrode solution was perfused at a pressure of 100 mmHg. Renal perfusion pressure was continuously monitored by a pressure cannula centered in the perfusion cannula and measured at the tip of the cannula. The organ chamber was warmed, and the inner cortical surface was continuously superfused with a Tyrode solution containing 1% albumin at 37°C and placed on the fixed stage of a Nikon Eclipse LV100 microscope. Afferent arterioles were then chosen for study, and diameter was measured using videomicroscopy.

Determination of afferent arteriolar diameter was accomplished using transillumination videomicroscopy as previously described. The tissue was transilluminated, and the focused image was converted to a video signal by a high-resolution Newvicon camera. Afferent arteriolar inside diameters were measured at 15-s intervals using a digital image-shearing monitor that yields measurements reproducible within 0.5 μm. The average diameter during the final 2 min of each 5-min treatment period was used for statistical analysis of steady-state responses.

Afferent arteriolar responses to acetylcholine 1, 3, and 6 wk following irradiation. Afferent arterioles were prepared for evaluation 1, 3, or 6 wk following irradiation. After a 20-min equilibration period, baseline diameter measurements of the afferent arteriole were obtained. Norepinephrine (0.5 μM) was added to the perfusate to elevate basal vascular tone. Acetylcholine (0.01–10 μM) was added to the perfusate, and vessel diameter changes were monitored for 5 min. Endothelial-independent relaxation was assessed at the end of experiment using sodium nitroprusside (100 μM).

NOS, COX, and EPOX pathways in the afferent arteriolar dilator responses. Three weeks following irradiation afferent arterioles were prepared for evaluation and compared with afferent arterioles from nonirradiated rats. Baseline diameter measurements of the afferent arteriole were obtained following a 20-min equilibration period. Norepinephrine (0.5 μM) was added to the perfusate to elevate basal vascular tone. NOS, COX, and EPOX inhibitors were then superfused for 20 min to ensure complete tissue blockade (20). Diameters were measured, and the concentration-response curve for acetylcholine were determined in the presence of inhibition. Inhibitors tested alone or in combination were the nitric oxide synthase inhibitor N5-nitro-l-arginine methyl ester (l-NAME; 100 μM), the EPOX inhibitor N-methylsulfonyl-6-(2-propargyloxyphenyl)hexanamide (MS-PPOH; 10 μM), or the COX inhibitor indomethacin (10 μM) (20). Acetylcholine (0.01–10 μM) was added to the superfusate, and vessel diameter changes were monitored for 5 min.

Angiotensin II and ATP afferent arteriolar constrictor responses. Three weeks following irradiation, afferent arteriolar responses to angiotensin II and ATP were compared with afferent arteriolar responses from nonirradiated rats. Baseline diameter measurements of the afferent arteriole were obtained following a 20-min equilibration period. After control diameter measurements, angiotensin II (0.1–10 nM) or ATP (0.1–100 μM) was delivered by superfusion, and a concentration-response curve was obtained.

Urine and plasma biochemical analysis. At the end of the 3-wk experimental period, urine was collected from rats housed in metabolic cages for 24 h. The following day, rats were anesthetized with isoflurane, and blood samples were collected from the abdominal aorta. Samples were then quickly frozen in liquid nitrogen and kept in a −80°C freezer until assayed for EET levels. Urinary levels of oxypapagene metabolites of arachidonic acid were determined using an LC-MS/MS method. Samples were warmed to room temperature and dried in a stream of nitrogen, and the residue was reconstituted in 40 μl of acetonitrile. Components were resolved on a 250 × 2.0 mm Kromasil C18 column packed with 5-μm diameter particles having 100-Å pores. Gradient elution from 85% solvent A to 15% solvent B was used with eluant flow of 0.3 ml/min. Solvent A was water with 0.01% formic acid, and solvent B was acetonitrile with 0.01% formic acid using the following profile: 15–30% solvent B in 10 min, 30–60% solvent B in 20 min, 60–80% solvent B in 15 min, hold at 80% for 5 min, and then 20 min reequilibration. MS/MS analysis was performed on an Agilent 6460 triple-quadrupole mass spectrometer equipped with a Jet Stream interface. Selected reaction monitoring was used to monitor oxidized arachidonate species in the negative ion mode. Precursor ion, product ion, collision energy, and fragmenter voltage were optimized for each compound. Other parameters were as follows: drying gas flow = 10 l/min at 325°C, nebulizer = 20 pounds/s, sheath gas flow = 11 l/min at 325°C, capillary = 3.5 kV, and nozzle = 1.0 kV. Results were acquired at unit-mass resolution.

Urinary biochemical analysis was done using colorimetric and ELISA assays. Oxidative stress was assessed by measurement of thiobarbituric acid (TBARS) and 8-isoprostane. Urinary TBARS and...
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urinary 8-isoprostane levels were measured by using commercially available assay kits (Cayman Chemical). Total nitric oxide production (from NOx = NO2 + NO3) was measured in urine using the Griess reaction and by a commercially available assay kit (Cayman Chemical).

Statistics. Data are presented as means ± SE. The significance of differences in mean afferent arteriolar diameter values between groups was evaluated with two-way analysis of variance for repeated measures followed by Duncan’s multiple-range test. The significance of differences in kidney microvessel EET levels was evaluated using an unpaired t-test. A value of P < 0.05 was considered statistically significant.

RESULTS

Overview data: Body weight, systolic blood pressure, BUN, and afferent arteriolar diameters. TBI results in decreased body weight gain, the development of renal injury, and hypertension 7–10 wk following TBI (4). In agreement with previous studies, rat body weight was decreased at 1 (control = 232 ± 6 g; n = 6 vs. TBI = 188 ± 5 g; n = 7), 3 (control = 262 ± 4 g; n = 20 vs. TBI = 207 ± 4 g; n = 21), and 6 (control = 274 ± 7 g; n = 6 vs. TBI = 212 ± 6 g; n = 6) wk following TBI. BUN (control = 16.7 ± 2.1 mg/dl; n = 6 vs. TBI = 19.1 ± 3.3 mg/dl; n = 6) and systolic blood pressure (control = 118 ± 7 mmHg; n = 6 vs. TBI = 121 ± 6 mmHg; n = 6) were unchanged in rats 6 wk following TBI. Baseline afferent arteriolar diameters were not different between control (21.9 ± 0.4 μm; n = 43) and TBI (21.5 ± 0.2 μm; n = 46) groups. Norepinephrine decreased afferent arteriolar diameters to a similar extent, and diameters averaged 15.2 ± 0.3 μm (n = 43) in control and 16.2 ± 0.4 μm (n = 46) after norepinephrine application in TBI.

Attenuated afferent arteriolar responses to acetylcholine in TBI rats. Afferent arteriolar responses to acetylcholine were assessed in control nonirradiated rats and TBI rats at 1, 3, and 6 wk following radiation exposure (Fig. 1). Acetylcholine-mediated afferent arteriolar dilation was similar in the 1-, 3-, and 6-wk control groups, and afferent arteriolar diameter increased by 25–33% to 10 μM acetylcholine. TBI resulted in a progressive attenuation of the afferent arteriolar dose-dependent dilation to acetylcholine. Afferent arteriolar responses to 10 μM acetylcholine were attenuated in TBI rats by 6% at 1 wk, 14% at 3 wk, and 20% at 6 wk. Attenuated afferent arteriolar responses to acetylcholine were not due to an inability of the vascular smooth muscle to respond to nitric oxide. The nitric oxide donor sodium nitroprusside (10 μM) caused a similar increase in afferent arteriolar diameter in control (39.5 ± 2.7%, n = 10) and TBI (37.0 ± 3.1%, n = 13) groups. These data demonstrate that the afferent arteriolar response to acetylcholine is significantly reduced in TBI rats, and this reduction in the vasodilator response is due to impaired endothelial function.

Urinary EPOX, NOS, and COX metabolite excretion rates in TBI rats. This next set of studies was conducted at 3 wk because there was attenuation of the afferent arteriolar responses to acetylcholine throughout the dose range in this TBI group. Urinary NOS, COX, and EPOX metabolites were evaluated in control and TBI rats at 3 wk (Fig. 2). There was a significant decrease in urinary EET + DHET levels in the TBI group. EET + DHET levels were reduced by 33% in the 11-Gy TBI group. The relative amounts of regioisomeric EETs and DHETs did not differ between controls (14,15-EET + 14,15-DHET = 46%, 11,12-EET + 11,12-DHET = 30%, 8,9-EET + 8,9-DHET = 24%) and TBI (14,15-EET + 14,15-DHET = 40%, 11,12-EET + 11,12-DHET = 33%, 8,9-EET + 8,9-DHET = 27%). Urinary 20-hydroxyeicosatetraenoic acid (20-HETE: TBI, 14.8 ± 2.3 vs. control, 12.3 ± 4.2 pg/mg creatinine) and prostacyclin (PGI2: TBI, 114.3 ± 18.7 vs. control, 106.0 ± 30.7 pg/mg creatinine) levels were not different between control and TBI groups. Radiation did not alter urinary nitrate/nitrite or TBARS at 3 wk. Urinary 8-isoprostane levels also did not differ between control (5.4 ± 0.4 pg/day, n = 5) and TBI (5.2 ± 0.7 pg/day, n = 6) groups.

Fig. 1. Afferent arteriolar dilator responses to acetylcholine progressively decrease from 1 to 6 wk following irradiation. Left, afferent arteriolar responses to acetylcholine 1 wk following irradiation. Control group diameters averaged 22.7 ± 1.0 μm under basal conditions and 16.1 ± 0.9 μm after adding norepinephrine (0.5 μM) to the perfusate (n = 6). Irradiation [total body irradiation (TBI)] group diameters averaged 22.0 ± 1.4 μm under basal conditions and 16.8 ± 1.3 μm after adding norepinephrine (n = 6). Middle, afferent arteriolar responses to acetylcholine 3 wk following irradiation. Control group diameters averaged 22.0 ± 0.9 μm under basal conditions and 15.7 ± 0.9 μm after adding norepinephrine (n = 7). Irradiation group diameters averaged 21.0 ± 1.0 μm under basal conditions and 16.1 ± 1.0 μm after adding norepinephrine (n = 11). Right, afferent arteriolar responses to acetylcholine 6 wk following irradiation. Control group diameters averaged 23.8 ± 1.3 μm under basal conditions and 15.7 ± 0.4 μm after adding norepinephrine (n = 5). Irradiation group diameters averaged 23.4 ± 1.0 μm under basal conditions and 17.4 ± 0.9 μm after adding norepinephrine (n = 5). Values are expressed as means ± SE. *Significant difference from the dose response to acetylcholine in the control group.

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These data demonstrate decreased epoxygenase levels but no change in urinary 20-HETE, nitric oxide, PGI2, or reactive oxygen species 3 wk following irradiation.

**Contribution of NOS, EPOX, and COX pathways in afferent arteriolar dilatation in TBI rats.** Figure 3 depicts the effect of EPOX, combined NOS and COX, and combined NOS, COX, and EPOX inhibition on the afferent arteriolar dilation to acetylcholine in control (left) and TBI (right) at 3 wk. As depicted in Fig. 1, the afferent arteriolar response to acetylcholine was significantly attenuated in TBI rats at 3 wk. Combined NOS, COX, and EPOX inhibition eliminated the afferent arteriolar acetylcholine dilator response in the 3-wk control and TBI groups. The contribution of the NOS and COX pathways to the afferent arteriolar dilation was similar between 3-wk control and TBI groups. Combined NOS and COX inhibition decreased the response to 10 μM acetylcholine by 16% in the control group and by 18% in the TBI group. This decrease was primarily NOS mediated because l-NAME alone decreased the response to 10 μM acetylcholine by 19% in the control group and by 16% in the TBI group. On the other hand, EPOX inhibition had a significantly different effect on the afferent arteriolar dilator response to acetylcholine in 3-wk control and TBI groups. MS-PPOH decreased the response to 10 μM acetylcholine from 130 ± 2 to 119 ± 4% in the control group and from 120 ± 2 to 119 ± 1% in the TBI group. These data demonstrate a decreased contribution of the EPOX pathway to the acetylcholine-mediated afferent arteriolar dilator response 3 wk following TBI.

**Afferent arteriolar constrictor responses to angiotensin II and ATP in TBI rats.** Afferent arteriolar responses to angiotensin II and ATP in 3-wk control and TBI groups are depicted in Fig. 4. The afferent arteriolar response to angiotensin II was enhanced at 3 wk in the TBI group. In contrast, the vasoconstrictor response to ATP was not altered in the 3-wk TBI group. These data are in agreement with previous studies demonstrating increased afferent arteriolar responses to angiotensin II and no change in the response to ATP when EET generation is decreased or inhibited (19, 47).

**DISCUSSION**

Irradiation is given before HSCT and has been clearly linked to life-threatening chronic kidney disease (8, 9, 37). After irradiation, there is a latent period of weeks to months before the development of clinical signs of hypertension and kidney disease (8, 37). Previous studies have demonstrated endothelial dysfunction and altered renal hemodynamics that precede radiation-induced kidney disease and hypertension (1, 3, 34, 38). The present study evaluated afferent arteriolar endothelial-dependent dilator responses at 1, 3, and 6 wk following TBI. We found a progressive attenuation in the afferent arteriolar dilation to acetylcholine but no change in the dilator response...
to nitroprusside. These data support the concept that afferent arteriolar endothelial dysfunction occurs in TBI before development of proteinuria, azotemia, or hypertension. Additional studies were conducted at 3 wk following irradiation to determine alterations in endothelial factors that contribute to the endothelial dysfunction. These experiments determined that attenuated afferent arteriolar dilation to acetylcholine 3 wk following TBI was due to decreased EPOX activity and EET levels. Therefore, afferent arteriolar endothelial dysfunction involves decreased EET levels that precede the development of proteinuria, azotemia, and hypertension in irradiated rats.

Our findings of a progressive decline in afferent arteriolar endothelial function are in accord with previous studies demonstrating impaired endothelial dilator responses and altered renal hemodynamics following irradiation (12, 23, 26, 38, 39). Afferent arteriolar dilator responses to acetylcholine were attenuated as early as 1 wk following irradiation. Juncos et al. evaluated renal dilator responses in rats 8 days following irradiation (30 Gy) and found that increases in renal blood flow were blunted in response to acetylcholine and bradykinin but not the nitric oxide donor nitroprusside (23). These findings support the notion that renal endothelial dysfunction following irradiation is an early event. A number of other studies have provided evidence that microvascular endothelial dysfunction is an early event and could contribute to the development of radiation injury in various organ systems (3, 12, 25, 29, 39). Endothelial-dependent dilation was impaired in human cervical arteries in patients 4–6 wk following irradiation (39). Interestingly, endothelial dysfunction in these patients receiving radiotherapy occurred without significant morphological damage to the endothelium. Vascular injury and morphological changes in the vasculature appear at later stages following irradiation. Overt renal vascular injury and renal arteriolar rarefaction occur at 20 wk after irradiation, and morphological vascular injury is a late radiation effect (2, 11, 30, 31). Taken together, these findings are in agreement with our findings in the afferent arterioles that endothelial- but not vascular smooth muscle-mediated dilation is impaired before clinical signs of kidney injury and hypertension.

Earlier studies have investigated the contribution of endothelial-derived factors following irradiation but have failed to do a complete comparison of the major endothelial metabolites. The current study evaluated the contribution of the NOS, COX, and EPOX pathways to the acetylcholine afferent arteriolar dilator responses in control and TBI rats. We found that the afferent arteriolar dilator response in control rats was largely dependent on NOS and EPOX pathways with a much smaller COX contribution. These data confirm a number of previous studies showing that afferent arteriolar dilation to acetylcholine or bradykinin is mediated by nitric oxide and EETs (19, 42, 45). Unlike the afferent arteriolar response in the control group, acetylcholine dilator responses in 3-wk TBI rats were not altered by EPOX inhibition. On the other hand, NOS inhibition attenuated the afferent arteriolar dilator response to acetylcholine to a similar extent in control and TBI rats. The decrease of EPOX-mediated but not NOS-mediated afferent arteriolar dilatation to acetylcholine is further supported by biochemical data demonstrating decreased urinary EETs but no change in nitric oxide or COX metabolites in TBI. Overall these findings provide strong evidence for a decrease in EPOX metabolites that contributes to endothelial dysfunction 3 wk following 11 Gy TBI in rats.

Previous studies evaluating endothelial-dependent dilator responses in irradiation have focused on nitric oxide or reactive oxygen species (12, 23, 38, 39, 40, 43, 46). Contributions for reactive oxygen species and decreased nitric oxide-dependent dilator responses have been observed in the intestinal microvasculature (12, 38, 44). Hatoum et al. observed increased intestinal microvascular reactive oxygen species production and superoxide dismutase mimetics improved endothelial-dependent relaxation in irradiated rats (12). Likewise, renal blood flow responses to acetylcholine were greatly attenuated by NOS inhibition in control and blunted at the highest doses in irradiated rats (23). COX inhibition failed to significantly decrease the acetylcholine-mediated increase in renal blood flow in control or irradiated rats (23). Other studies have demonstrated that the COX metabolite PGI2 is decreased in cultured endothelial cells exposed to radiation (10, 13). Inter-
estingly, isolated glomeruli and cultures of glomerular epithelial and mesangial cells exposed to radiation demonstrate release of arachidonic acid and increased prostaglandin 
P_2_α and prostaglandin 
E_2 levels within 10 min (35). However, our data demonstrated no alteration in the afferent arteriolar response to acetylcholine in the presence of the COX inhibitor indomethacin in TBI rats. In addition, urinary 
P_G_2 levels were not altered 3 wk following TBI. Thus, the findings of our current study failed to demonstrate a significant contribution for reactive oxygen species, nitric oxide, or COX metabolites to impaired afferent arteriolar endothelial responses in irradiated rats.

The radiation dose in the current study was much lower than the doses in studies that showed decreased nitric oxide and increased reactive oxygen species. Our study used an 11-Gy TBI single fraction, whereas the previous studies used either a 30-Gy single fraction or three fractions per week on alternate days for three successive weeks totaling 22 Gy (12, 23). Biochemical analysis in the current study demonstrated no change in nitric oxide or reactive oxygen species. This finding is in agreement with previous studies that have failed to demonstrate chronically increased kidney oxidative stress in rats exposed to 11 Gy single-fraction TBI (7, 24). Likewise, urinary 
G_M_P levels were not altered at 6 wk but decreased at 9 wk following 17 Gy TBI given in six fractions, a dose that is equivalent to ~10 Gy TBI in a single fraction (6). Our current evaluation of NOS, COX, and EPOX pathways in rats exposed to 11 Gy TBI demonstrates impaired afferent arteriolar endothelial-dependent dilation that is due to decreased EPOX levels without a change in the nitric oxide dilator component. These data are relevant to clinically used radiation doses.

We extended the findings of our current study to include afferent arteriolar constrictor responses 3 wk following irradiation. These data revealed enhanced vasoconstrictor responses to angiotensin II but an unaltered ATP vasoconstrictor response. These results are consistent with previous findings in this model that showed exacerbation of renal radiation injury when angiotensin II was given by an osmotic pump from week 4 to week 8 after 17 Gy TBI in six fractions (5). Enhanced afferent arteriolar constrictor responses to angiotensin II have been previously observed early in the development of hypertension and in cases where there are decreased endothelial EPOX metabolite levels (15, 21). Our findings with angiotensin II and ATP are consistent with the afferent arteriolar responses that show endothelial dysfunction but no changes in vascular smooth muscle responses at 3 wk after TBI. Afferent arteriolar responses to ATP correlate with changes in the myogenic response (17). During hypertension, afferent arteriolar responses to ATP and myogenic constrictor responses are attenuated (17, 48). We have previously found that 12 wk following TBI afferent arteriolar responses to acetylcholine were impaired (14). Thus, endothelial dysfunction occurs before the development of azotemia, proteinuria, and hypertension in irradiated rats, whereas afferent arteriolar myogenic ATP constrictor responses are attenuated after the development of hypertension in TBI rats.

In summary, afferent arteriolar dilation to acetylcholine was progressively attenuated from 1 to 6 wk following irradiation in rats. We also show that afferent arteriolar dysfunction without a change in vascular smooth muscle function occurs before azotemia, proteinuria, and hypertension in TBI rats. Assessment of the three major endothelial dilator pathways revealed that a decrease in EPOX metabolites contributed to the afferent arteriolar endothelial dysfunction in TBI rats. In agreement with the findings of the current study, we recently demonstrated that an EET analog administered from 2 days to 12 wk following TBI improved afferent arteriolar responses to acetylcholine, decreased blood pressure, and reduced kidney injury (14). Taken together, these results indicate that afferent arteriolar endothelial dysfunction involves decreased EPOX levels that precede the development of proteinuria, azotemia, and hypertension in irradiated rats.

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DISCLOSURES

John D. Imig has patents related to epoxyenase pathway for the treatment of renal and cardiovascular diseases.

AUTHOR CONTRIBUTIONS


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