Arachidonic Acid Metabolites Mediate the Radiation-Induced Increase in Glomerular Albumin Permeability

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Radiation-induced renal injury is characterized by proteinuria, hypertension, and progressive decline in renal function. We have previously shown that *in vivo* or *in vitro* irradiation of glomeruli with a single dose of radiation (9.5 Gy) increases glomerular albumin permeability (P_{alb}) within 1 hr. The current studies tested the hypothesis that this early radiation-induced increase in P_{alb} is caused by the release of arachidonic acid and by the generation of specific arachidonic acid metabolites. Glomeruli obtained from WAG/Rij/MCW rats and cultured rat glomerular epithelial and mesangial cells were studied after irradiation (9.5 Gy, single dose). Arachidonic acid release and eicosanoid synthesis by glomeruli or cultured glomerular cells were measured after irradiation, and the effect of inhibitors of phospholipase A_2 (PLA_2) and cyclooxygenase (COX) on the irradiation-induced increase in P_{alb} was assessed. Arachidonic acid release was demonstrated within 10 mins of irradiation of isolated glomeruli and monolayer cultures of glomerular epithelial and mesangial cells. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and PGE_2 release was increased after irradiation of isolated glomeruli. Blocking arachidonic acid release or COX activity before irradiation completely prevented the increase in P_{alb}. COX inhibition immediately after irradiation also diminished the radiation-induced increase in P_{alb}. We conclude that arachidonic acid and its COX metabolites play an essential role in the early cellular changes that lead to the radiation-induced increase in P_{alb}. Understanding of the early epigenetic effects of irradiation may lead to new intervention strategies against radiation-induced injury of normal tissues. Exp Biol Med 231:99–106, 2006

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Introduction

Radiation-induced renal injury (radiation nephropathy) is characterized by proteinuria, hypertension, and progressive decline in renal function (1). It is seen in the setting of total body irradiation (TBI) in preparation for bone marrow transplantation (2) but can also be observed after external beam therapeutic irradiation (3) and after therapeutic use of radiolabeled biologicals (4). Additionally, renal injury could occur as a result of radiation accidents or radiologic terrorism (5, 6). Radiation nephropathy develops months to years after irradiation, but the early cellular events that contribute to the appearance of clinical symptoms such as overt proteinuria are not clearly understood (1, 2, 7).

We have previously shown that radiation-induced renal failure in the rat can occur as early as 8 months after a single dose of 8.7 Gy and that renal dysfunction (e.g., elevated BUN) can be observed by 7 months after a single dose of 6.5 Gy (8). In the same strain of rats, *in vivo* irradiation with a single dose of 9.5 Gy increases glomerular albumin permeability (P_{alb}) at 1 hr, even though rats irradiated in this protocol developed proteinuria only after 4 weeks (2). P_{alb} is increased in a dose-dependent manner in glomeruli irradiated *ex vivo* at 5.5–9.5 Gy; this dose-dependent increase in P_{alb} is correlated (Kendal $\tau = 0.84$, $P = 0.04$) with the azotemia that develops later in rats irradiated in this protocol (7). This increase in P_{alb} may serve as an indicator of initial changes in the glomerular filtration barrier and of the chronic renal injury that appears following renal or total body irradiation (1–7).

Ionizing radiation increases the release of arachidonic acid from membrane phospholipids and the generation of eicosanoids through up-regulation of phospholipase A_2 (PLA_2) (9) and cyclooxygenase (COX) (10). Eicosanoids

are known for their vascular effects and for their role in injury and inflammation (11–13). We hypothesized that radiation-induced release of arachidonic acid and/or radiation-induced generation of eicosanoids causes increased glomerular albumin permeability (P_{alb}). Results of our initial test of this hypothesis and its relevance to the prevention and mitigation of radiation-induced renal disease are presented in this communication.

Materials and Methods

Experimental Model. WAG/Rij/MCW rats were maintained on sterilized rat chow in a moderate-security barrier facility at the Biomedical Resource Center of the Medical College of Wisconsin (MCW). In all cases, kidneys were removed via abdominal incision after the animals were anesthetized using halothane (Halocarbon Laboratories, River Edge, NJ). Animal care was in accordance with NIH guidelines, and the Animal Care and Use Committee at the Medical College of Wisconsin approved of all protocols. All irradiations were carried out with a single dose of 9.5 Gy. This radiation dose induces a robust (and near maximal) effect on glomerular permeability (P_{alb}) (7) but is low enough that long-term *in vivo* studies can be carried out in a bone transplant model without causing acute radiation injury to other organ systems (8).

Arachidonic Acid Release from Isolated Glomeruli. Kidneys were removed from 8- to 9-week-old rats, and glomeruli were isolated in RPMI-1640 (Gibco, Invitrogen Corporation, Carlsbad, CA) by established sieving techniques (7). Glomeruli from both kidneys were pooled to obtain one sample from each rat. Isolated glomeruli were incubated for 60 mins with 16.7 kBq [^{3}H]arachidonic acid (Amersham Pharmacia Biotech, Piscataway, NJ) in RPMI-1640 (Gibco) containing 0.1% bovine serum albumin (BSA, Sigma Chemical Company, St. Louis, MO). The incubator atmosphere was maintained at 37°C and 5% CO_2. Glomeruli were washed with fresh medium without radioactivity and incubated for 30 mins to allow distribution and equilibration of the labeled arachidonic acid. At the end of the equilibration period, glomeruli were washed with RPMI-1640.

The experimental group was irradiated with 9.5 Gy in a ^{137}Cs γ-ray generator (Model 137; JL Shepherd, San Fernando, CA). Sham-irradiated glomeruli were used as the control group. Glomeruli incubated with 5 μg/ml calcium ionophore A-23187 (Sigma Chemical Company) served as the positive control. Medium was collected at 10 or 60 mins posttreatment and mixed with 6 volumes of ethyl acetate acidified by 1 *M* formic acid (0.08% v/v). Radioactivity in the ethyl acetate extract was measured using a liquid scintillation counter (LKB RackBeta 1214, Pharmacia, Wallac Oy, Finland). Total protein in glomerular preparation and cells was determined by Lowry's method (14) using a reagent kit (Bio-Rad, Hercules, CA). The measured radioactivity was expressed as counts per minute (cpm)/mg total protein.

Monolayers of Glomerular Epithelial Cells and Mesangial Cells. Rat glomerular epithelial cells (passage 10–14) were grown to confluence on 10-cm tissue culture petri dishes in K1 medium containing 2% Nu Serum (Collaborative Biomedical Products, Becton Dickinson, Bedford, MA). Rat mesangial cells (passage 10–14) were grown to confluence in RPMI-1640 containing 15% fetal bovine serum (FBS, Gibco) and maintained in RPMI-1640 containing 10% FBS. Confluent cultures were washed with serum-free RPMI-1640 and incubated with 10^6 cpm [^{3}H]arachidonic acid in RPMI-1640 containing 0.1% BSA for 60 min. Cells were washed with fresh radioactivity-free RPMI-1640 and incubated for 30 mins to allow equilibration of the radioactive arachidonic acid. At the end of the equilibration period, cells were washed with fresh RPMI-1640, divided into three groups, and irradiated with 9.5 Gy (^{137}Cs γ-ray), sham treated, or incubated with calcium ionophore (5 μg/ml). Each petri dish was used as one sample. Aliquots of the media were collected at 10 mins after irradiation. Released radioactivity was extracted with acidified ethyl acetate and measured as described in the previous section.

Competitive Enzyme Immunoassay (EIA) of Prostaglandin E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$). We have previously shown that PGE_2 or $PGF_{2\alpha}$ induced an increase in P_{alb} (14) and therefore selected these two COX products of arachidonic acid for analysis in the present studies. Isolated glomeruli were irradiated or sham-irradiated as described above. Aliquots of media at 5 or 15 mins after irradiation were processed for EIA of PGE_2 or $PGF_{2\alpha}$ according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI). Total protein was determined by Lowry's method (14), and the concentration of eicosanoids expressed as picograms per milligram protein.

Treatment of Isolated Glomeruli with Inhibitors. Isolated glomeruli were incubated with the PLA_2 inhibitor quinacrine (10 μ*M*, Sigma Chemical Company) or a COX inhibitor (see below) and irradiated with 9.5 Gy (^{137}Cs γ-rays), and P_{alb} was determined at 60 mins postirradiation. The COX inhibitors assessed were indomethacin (1 μ*M*, Sigma Chemical Company), acetylsalicylic acid (aspirin, 1.25 m*M*, Sigma Chemical Company), and ibuprofen ([±]-2-[4-isobutylphenyl]-propionic acid, 20 μ*M*, Cayman Chemical) as they are the most commonly used experimental and clinical COX inhibitors. We have previously shown that indomethacin can effectively prevent an increase in P_{alb} generated by other agents (15). Inhibitor-treated glomeruli without irradiation were used as control. Sham-irradiated control glomeruli were preincubated with 10 μL of ethanol, the vehicle. Irradiated glomeruli were used as positive control. All incubations were carried out at 37°C for 10 mins in 1 ml final volume made with a physiological buffer containing 5% BSA.

In a separate series of experiments, isolated glomeruli were incubated with ibuprofen (20 μM) for 30 mins before or immediately after (within 2 mins) irradiation (9.5 Gy ^{137}Cs γ-rays), and P_{alb} was measured after 60 mins. Control groups consisted of glomeruli treated with or without ibuprofen alone. This experimental design was used to allow us to assess whether the COX inhibitors were working via a "mitigation" mechanism as opposed to a "protection" mechanism. We selected ibuprofen instead of indomethacin and acetylsalicylic acid because ibuprofen inhibits COX-1 and COX-2 at about equimolar concentrations (16), and it is the most commonly used nonsteroidal antiinflammatory agent. Such distinction is not critical for potential applications in therapeutic irradiation but is critical for application to radiation accidents or radiologic terrorism that may affect a very large number of people in a short time (5, 6).

Measurement of Glomerular Convectional Permeability to Albumin. The *in vitro* assay of albumin permeability indicates changes in the characteristics of the glomerular filtration barrier that restricts macromolecules (colloids) to plasma. The capillary plasma pressure attributed to macromolecules is called oncotic pressure or colloidal osmotic pressure. Lowering the extracapillary oncotic pressure can generate an oncotic gradient that would result in a movement of fluid into the capillary. The volume response of glomerular capillaries to an oncotic gradient was measured as previously described (7). In brief, following the experimental manipulations as described above glomeruli were transferred to a glass cover slip coated with poly-L-lysine (1 mg/ml, Sigma Chemical Company) and observed using videomicroscopy before and about 1 min after the medium containing 5% BSA was replaced by medium containing 1% BSA. This exchange of medium produces an oncotic gradient across the glomerular capillary wall and results in a net influx of fluid and an increase in glomerular volume. The increase in volume (ΔV) of each glomerulus in response to an oncotic gradient was expressed as: $\Delta V = (V_{final} - V_{initial})/V_{initial} \times 100\%$.

There is a direct relationship between the increase in glomerular volume (ΔV) and the oncotic gradient ($\Delta \Pi$) applied across the capillary wall. We used this principle to calculate σ_{alb}, using the ratio of ΔV of experimental to ΔV of control glomeruli in response to identical oncotic gradients: $\sigma_{alb} = \Delta V_{experimental}/\Delta V_{control}$.

Convectional albumin permeability (P_{alb}) was defined as $(1 - \sigma_{alb})$ to describe the movement of albumin consequent to water flow. When σ_{alb} is zero, albumin moves at the same rate as water, and P_{alb} is 1.0. Alternatively, when σ_{alb} is 1.0, albumin cannot cross the membrane with water, and P_{alb} is zero.

Statistical Analysis. P_{alb} values are expressed as mean ± SEM. Values of groups were compared using *t* test. Significance of difference between groups was expressed as *P* value. Selected data were rechecked and reconfirmed by nonparametric analysis using the Mann-Whitney test.

Results

Release of Arachidonic Acid from Rat Glomeruli. Irradiation of glomeruli with a 9.5-Gy single dose resulted in increased release of arachidonic acid and its metabolites at 10 mins (both $P < 0.001$; Fig. 1). Radioactivity in the medium from the calcium ionophore-treated positive control group at 10 mins and 60 mins was comparable to that observed in the irradiated group. The released arachidonic acid in the irradiated and ionophore-treated groups at 60 mins was 57% and 66% of that at 10 mins. The sham-irradiated control groups at 10 and 60 mins were not different from each other ($P > 0.1$; Fig. 1).

Release of Arachidonic Acid from Irradiated Monolayers of Glomerular Epithelial Cells and Mesangial Cells. Irradiation of cultured glomerular epithelial cells (Fig. 2A) and mesangial cells (Fig. 2B) with a 9.5-Gy single dose resulted in increased release of arachidonic acid and its metabolites at 10 mins (both $P < 0.001$). Irradiation caused a 2.2-fold increase in released radioactivity from glomerular epithelial cells and mesangial cells, whereas the ionophore-induced increase in the released radioactivity was 5.2- and 7.4-fold from glomerular epithelial cells and mesangial cells, respectively (Fig. 2).

Release of PGE_2 and $PGF_{2\alpha}$ from Irradiated Glomeruli. PGE_2 synthesized by irradiated glomeruli was 45% higher than that by control glomeruli at 15 mins ($P < 0.001$), but the difference was not significant ($P > 0.10$) at 5 mins (Fig. 3A). $PGF_{2\alpha}$ synthesized by irradiated glomeruli at 15 mins showed a 145% increase over the control group ($P < 0.001$), but the difference was not significant ($P > 0.10$) at 5 mins (Fig. 3B).

Effect of Inhibitors of Arachidonic Acid Metabolism on the Irradiation-Induced Increase in P_{alb}. The P_{alb} of groups treated with quinacrine (Q) alone, indomethacin (I) alone, or acetylsalicylic acid (A) alone were not different from that of the sham-irradiated control (all $P > 0.10$; Fig. 4). Pretreatment with quinacrine, indomethacin, or acetylsalicylic acid blocked the radiation-induced increase in P_{alb} (all $P < 0.001$ vs. the Rad alone group; Fig. 4).

Ibuprofen also protected the glomerular permeability barrier from radiation injury (Fig. 5). Addition of ibuprofen before as well as immediately after irradiation alleviated the radiation-induced increase in P_{alb} (both $P < 0.001$; Fig. 5), but treatment before irradiation was more effective than treatment after irradiation.

Discussion

We demonstrate for the first time that a radiation-induced increase in glomerular albumin permeability can be prevented by inhibiting the cyclooxygenase pathway of arachidonic acid metabolism. Irradiation causes rapid release of arachidonic acid from both isolated intact glomeruli and cultured glomerular cells. Furthermore, glomerular synthesis of PGE_2 and $PGF_{2\alpha}$ was increased

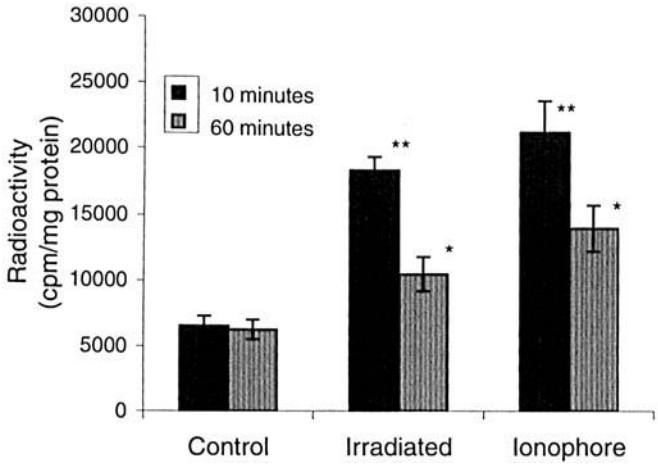

Figure 1. Release of arachidonic acid from irradiated rat glomeruli. Isolated glomeruli were preincubated with [^{3}H]arachidonic acid for 1 hr. After a 30-min equilibration, glomeruli were irradiated (9.5 Gy, single dose), incubated with calcium ionophore (5 μg/ml), or sham-irradiated. Arachidonic acid and metabolites were extracted from media at 10 or 60 mins posttreatment, and total radioactivity was measured. Glomeruli from two kidneys of the same animal were pooled to obtain one sample (8 samples in each group). Data are shown as the means ± the standard error. Significant differences from pooled sham-irradiated controls are marked (*$P < 0.01$ and **$P < 0.001$).

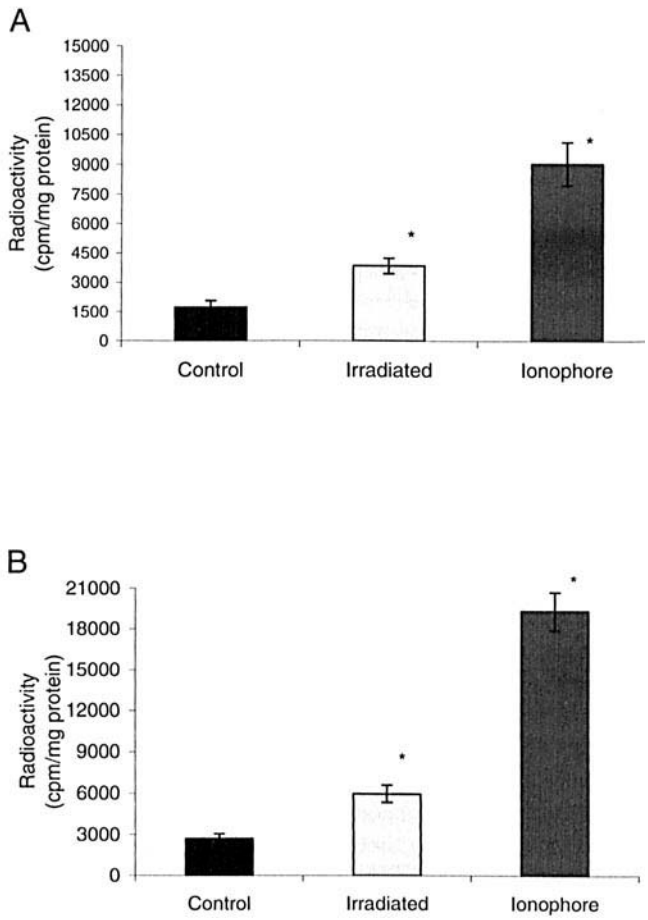

Figure 2. Release of arachidonic acid from irradiated monolayers of glomerular epithelial cells and mesangial cells. Confluent cultures of glomerular epithelial cells (A) or mesangial cells (B) were preincubated with [^{3}H]arachidonic acid for 1 hr. After equilibration for 30 mins, cells were irradiated (9.5 Gy, single dose), incubated with calcium ionophore (5 μg/ml), or sham-treated. Arachidonic acid and metabolites were extracted from the medium at 10 mins posttreatment. (A) Total radioactivity released from control ($n = 8$), irradiated ($n = 7$), and ionophore-treated ($n = 4$) experiments with glomerular epithelial cells. (B) Total radioactivity released from control ($n = 8$), irradiated ($n = 8$), and ionophore-treated ($n = 5$) mesangial cells. Data are shown as the means ± the standard error. Significant differences from sham-irradiated controls are marked (*$P < 0.001$).

immediately following irradiation. Inhibition of the release and metabolism of arachidonic acid blocked the increase in P_{alb}, supporting our hypothesis that one or more eicosanoids mediate the early glomerular changes that precedes proteinuria in a model of radiation nephropathy.

Proteinuria is associated with several cardiovascular diseases and is one of the major problems in modern medicine. It reflects a fairly advanced stage of renal damage that is compounded by the impact of the protein in the glomerular filtrate on tubular function and by the effects of progressive increase in urinary protein (17–19). Clinical radiation nephropathy is also characterized by proteinuria, azotemia, and hypertension that develop months to years after irradiation (3, 20). In a rat model of radiation nephropathy, overt proteinuria develops only after about 4 weeks (1). The latency before the development of proteinuria in radiation nephropathy offers an opportunity to explore the initial changes that lead to pathophysiologic symptoms and organ failure so as to intervene in disease progression.

We have reported that although P_{alb} increases within 1 hr after irradiation, receptor-mediated tubular binding of albumin deteriorates only several weeks later (21), indicating a difference in the effect of radiation on glomeruli and tubules. We have used *in vitro* determination of P_{alb} as a marker of preproteinuric glomerular changes in the Zucker obese rats (22), puromycin aminonucleoside (PAN)-induced nephrosis (23), dietary salt-induced proteinuria with hypertension (24), and recurrent focal segmental glomerulosclerosis (FSGS) (25). In each of these models, glomerular injury (as indicated by increased P_{alb}) precedes the onset of proteinuria, suggesting that *in vitro* glomerular permeability is a sensitive and reliable marker of the initial injury to the glomerular filtration barrier. Identification of early markers of glomerular and tubular injury is essential for effective management and treatment of radiation-induced renal dysfunction (6).

The immediate increase in glomerular permeability following irradiation reflects rapid metabolic events affecting the structure and function of the glomerular protein permeability barrier and is less likely to reflect altered gene expression at this time point. Such epigenetic processes may trigger molecular pathways that alter biological characteristics in a tissue/cell-specific manner and result in gene activation (26). We postulate that immediate responses to injurious agents or environmental changes may involve membrane- and cytoskeleton-associated molecules and

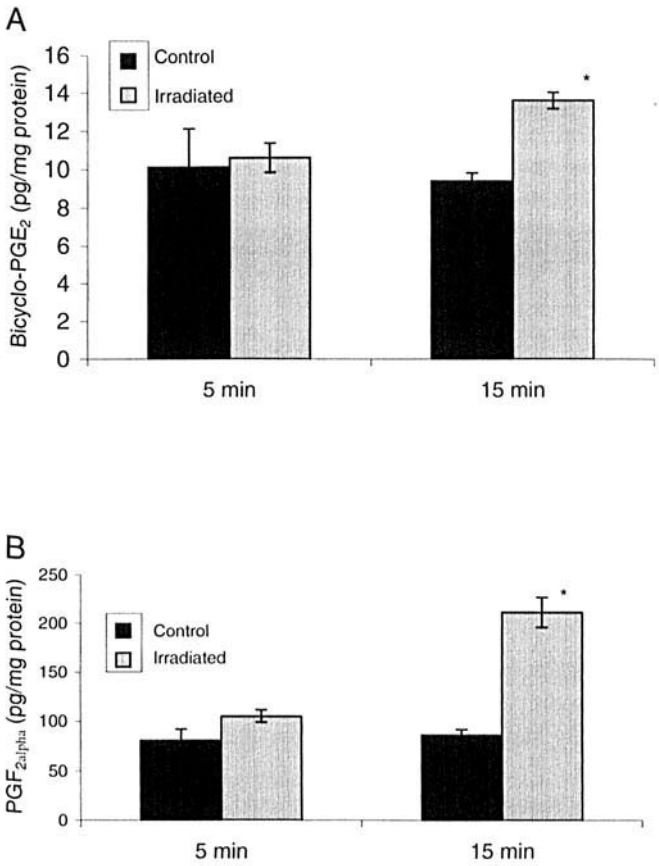

Figure 3. PGE_2 and $PGF_{2\alpha}$ release from isolated irradiated glomeruli. Isolated glomeruli were irradiated (9.5 Gy, single dose) or sham-irradiated. Released eicosanoids were extracted from media at 5 or 15 mins after irradiation, and PGE_2 (A) and $PGF_{2\alpha}$ (B) were determined by EIA. Glomeruli from two kidneys of the same animal were pooled to obtain one sample (n = 3 samples in each group). Data are shown as the means ± standard error. Significant differences from concurrent sham-irradiated controls (*$P < 0.001$).

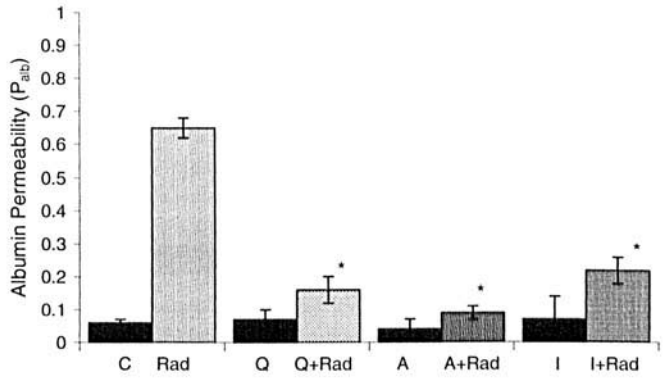

Figure 4. Effect of inhibitors of arachidonic acid metabolism on the radiation-induced increase in P_{alb}. Isolated glomeruli preincubated with quinacrine (Q), indomethacin (I), or acetylsalicylic acid (A) were either used as control (Q, I, and A, 15 samples in each group) or after irradiation (Q+Rad, I+Rad, and A+Rad, 15 samples in each group). Sham-treated glomeruli were used as baseline control (C, 30 samples), and irradiated glomeruli without drug treatment were used as positive control (Rad, 20 samples). P_{alb} was determined at 1 hr postirradiation. Results are shown as the means ± the standard error. Significant differencest from animals treated with radiation alone are marked (*$P < 0.001$).

signaling pathways. Arachidonic acid, a polyunsaturated essential fatty acid in membrane phospholipids, and its metabolites may play key roles in the epigenetic events that lead to increased permeability.

In the present study a single dose of ionizing radiation increased the release of arachidonic acid from glomeruli (Fig. 1) as well as from glomerular cells (Fig. 2). The finding that arachidonic acid in the medium was lower at 60 mins after irradiation than at 10 mins suggests a dynamic exchange of arachidonic acid between the medium and glomerular cells in the intervening period. The immediate release of arachidonic acid by glomeruli and glomerular cells after irradiation is in marked contrast to the response of cultured HL-60 cells (a leukemia cell line) in which arachidonic acid release occurred only at about 30 mins after irradiation (27). The difference between the radioactivity released from whole glomeruli and cultured glomerular cells indicates differences between cells within the glomerulus. Thus, the release of arachidonic acid in response to irradiation appears to occur in a tissue- or cell-specific manner that depends on the composition of the membrane. The characteristic features of cellular membrane such as conductance, fluidity, and permeability are determined by its protein and lipids, which, in turn, are determined by the chain length and the degree of unsaturation of fatty acids (29). Thus, the radiation-induced changes in the membrane composition and the increased levels of free arachidonic acid are likely to affect several cellular events. For example, arachidonic acid activates protein kinase C (PKC) *in vitro* (30–32) and thus influences the signaling events mediated by PKC. These changes may occur without invoking DNA-dependent synthesis of response molecules. Our findings suggest a role for arachidonic acid and its metabolites in the early cellular signaling changes that lead to increased P_{alb}.

Nonesterified free arachidonic acid is metabolized through the COX, lipoxygenase, and cytochrome P-450 pathways into several eicosanoids that are important in vascular regulation and tissue response to injury (Fig. 6). Earlier reports indicate that total body irradiation (TBI) of mice or rats increases urinary prostaglandins and thromboxane B_2, indicating a role for these metabolites in the immediate response to radiation (33–37). Changes in urinary PGE_2 after TBI were found to follow a biphasic pattern (38). Some of eicosanoids may be of systemic origin because thoracic shielding of rats prevented urinary excretion of thromboxane B_2 (39) following TBI. Our results show that irradiation induces increased levels of PGE_2 and $PGF_{2\alpha}$ in glomeruli (Fig. 4).

The renal prostaglandins and thromboxanes act as autacoids to modulate renal hemodynamics and may have direct effects on the glomerular filtration barrier. We have recently shown that PGE_2 and $PGF_{2\alpha}$ cause increased glomerular albumin permeability of isolated glomeruli in the absence of neurohumoral or hemodynamic factors (15).

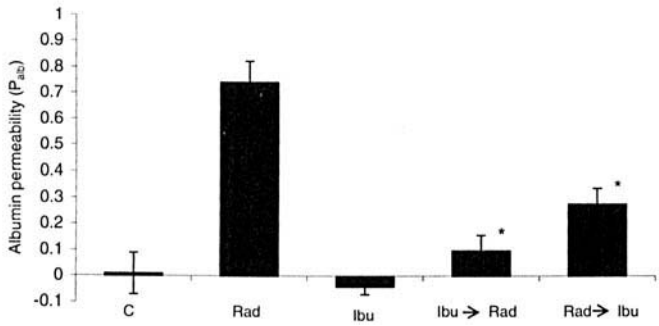

Figure 5. Effect of ibuprofen on the radiation-induced increase in P_{alb}. Glomeruli were sham-irradiated (C), given irradiation only (Rad), given ibuprofen only (Ibu), preincubated with ibuprofen for 30 mins followed by irradiation (Ibu → Rad), or irradiated and then incubated with ibuprofen for 60 mins (Rad → Ibu) (15 samples in each group). Data are shown as the means ± standard error. Significant differences from animals treated with irradiation only are marked (*$P < 0.001$).

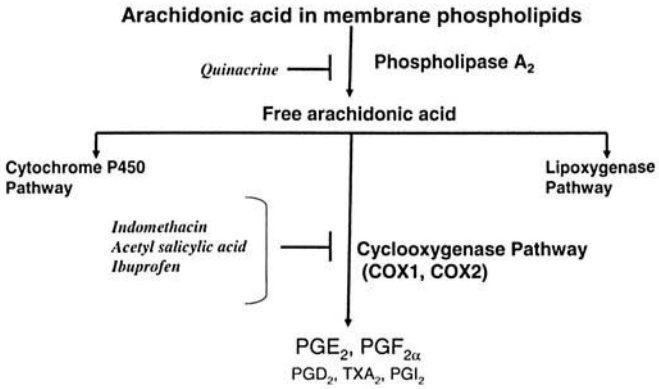

Figure 6. A simplified version of arachidonic acid metabolism. Cellular arachidonic acid is largely present in its esterified form with membrane phospholipids. Nonesterified arachidonic acid is generated by the action of phospholipase A_2 on phospholipids. The nonesterified form of arachidonic acid is metabolized *via* three main metabolic pathways involving cyclooxygenases, lipoxygenases, and cytochrome P450 enzymes. We have previously shown that cyclooxygenase products PGE_2 and $PGF_{2\alpha}$ cause increased glomerular albumin permeability (14).

Further, PGE_2 increases depolymerization of actin filaments in podocytes, indicating cytoskeletal changes that may alter glomerular filtration barrier function (40). Cellular effects of prostaglandins are receptor mediated. For example, PGE_2 binds to four E-prostanoid receptors (EP1–EP4), each of which preferentially couples to a different signal transduction pathway, including stimulation of cAMP generation by the EP2 and EP4 receptors; inhibition of cAMP generation *via* G_i by EP3 receptors, and activation of phosphatidylinositol hydrolysis by EP1 receptors. $PGF_{2\alpha}$ has been shown to bind to the FP receptor and cause mesangial cell contraction and proliferation through the phosphoinositide–phopholipase C and protein kinase C signaling mechanisms (41, 42).

Release of arachidonic acid from membrane phospholipids by PLA_2 and its metabolism into PGE_2 and $PGF_{2\alpha}$ by COX-1 and –2 increases following injury (28, 37, 43). Present studies also indicate the role of these enzymes in the radiation-induced increase in glomerular albumin permeability. Radiation-induced increase in PLA_2 and COX-2 has been demonstrated in cells such as astrocyte and microglial cultures (9, 44–46). Further evidence for the role of PLA_2 and COX in the cellular response to radiation injury is provided by pharmacologic inhibition or gene deletion. Treatment with NS-398, a COX-2 specific inhibitor, lowers the increased levels of PGE_2 in irradiated brain (46). In addition, the radiation-induced increase in PGE_2 in intestinal cells is attenuated by indomethacin (47), Tepoxalin (an inhibitor of COX-1, COX-2, and 5-lipoxygenase) (48), or by COX-1 gene deletion (10).

The present studies show that inhibitors of the COX pathway of arachidonic acid metabolism block the radiation-induced increase in glomerular P_{alb}. Because of the current interest in developing agents that will be effective for the mitigation and treatment of radiation injuries (5, 6), we further tested the efficacy of COX inhibitors given after irradiation (a mitigation protocol). We observed a significant protective effect of ibuprofen on radiation-induced increase in P_{alb} under both conditions, although pretreatment appears to be more effective than postirradiation, indicating the extremely rapid nature of changes induced by irradiation (Fig. 5).

Indomethacin, acetylsalicylic acid, and ibuprofen are clinically used NSAIDs that inhibit both COX-1 and –2 to varying degrees. The easily available S-(+) enantiomer of (±)-ibuprofen reversibly inhibits both isoforms of COX at about equimolar concentrations (ID_{50} 8.9–14.0 μM and 7.2–8.2 μM for COX-1 and COX-2, respectively) (16). Our findings strongly suggest that one or more COX metabolites of arachidonic acid participate in the early events that lead to increase in P_{alb} and may be important in the processes that lead to clinical disease weeks to months later in this model of radiation nephropathy.

Proteinuria is managed and treated using strategies that include cytotoxic agents, angiotensin-converting enzyme inhibitors (ACE), and nonsteroidal antiinflammatory drugs (NSAIDs). These regimens are aimed at retardation of the progress of proteinuria and the effects of increased protein in the glomerular filtrate. NSAIDs lower proteinuria in nephrotic syndrome resistant to corticosteroid and cytotoxic therapy (49–53). However, the mechanism by which this treatment limits glomerular filtration of proteins is not understood. In this regard, we have shown that indomethacin protects the glomerular filtration barrier from injury caused by some eicosanoids of the COX pathway (15). The present data also indicate that eicosanoids such as PGE_2 are involved in a radiation-induced increase in glomerular protein permeability.

In summary, our results suggest that metabolites of arachidonic acid contribute to the increase in glomerular permeability that is observed after a single dose of radiation. This finding points to a role for epigenetic phenomena in cellular responses to radiation. Traditionally, DNA damage

was believed to be responsible for all radiation-induced biological changes, including the immediate release of arachidonic acid (54). The importance of radiation-induced extranuclear signaling in gene expression is being increasingly acknowledged (26). We hypothesize that irradiation initiates cellular processes before the onset of genomic changes and that increased glomerular permeability is the functional manifestation of the early cellular responses to injury. Because clinical symptoms of renal injury appear weeks to months after irradiation and represent culmination of progressive changes in the entire kidney, detection of increased glomerular albumin permeability may be useful in developing strategies for early interventions. These preliminary findings also suggest that certain inhibitors of the arachidonic acid metabolism may be useful for both preventing and mitigating radiation injuries.

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