Acute and chronic microvascular alterations in a mouse model of ischemic acute kidney injury

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1Division of Nephrology, Department of Medicine and the Indiana Center for Biological Microscopy, Indiana University School of Medicine, Indianapolis, Indiana; 2Department of Nephrology, University Hospital, University Duisburg-Essen, Essen, Germany; and 3Division of Immunology, Shigei Medical Research Institute, Okayama, Japan

Submitted 13 November 2006; accepted in final form 10 July 2007

Hörbelt M, Lee S-Y, Mang HE, Knipe NL, Sado Y, Kribben A, Sutton TA. Acute and chronic microvascular alterations in a mouse model of ischemic acute kidney injury. Am J Physiol Renal Physiol 293: F688–F695, 2007. First published July 11, 2007; doi:10.1152/ajprenal.00452.2006.—Functional and structural abnormalities in the renal microvasculature are important processes contributing to the pathophysiology of ischemic acute kidney injury (AKI). In this study, we examine the contribution of endothelial cell loss via apoptosis on microvascular permeability and rarefaction in a mouse model of ischemic AKI. Three-dimensional reconstructions of microvascular networks obtained 24 h following acute ischemic injury demonstrate an intact endothelial monolayer in areas of increased microvascular permeability. A 45% decrease in microvascular density was observed 4 wk after acute ischemic injury. Examination of microvascular endothelial cells following acute ischemic injury did not reveal evidence of positive terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling staining at 1, 2, 8, and 16 days following ischemia; however, activation of caspase-3 was evident in endothelial cells following acute ischemic injury. Examination of angiopoietin (Ang) protein expression in the kidney 24 h after ischemic injury revealed an eightfold increase in Ang-1 but no significant change in Ang-2. No significant difference in the expression of vascular endothelial growth factor or Ang-2 was observed 4 wk after ischemic injury, although an almost twofold elevation in Ang-1 was observed. An increase in angiostatic breakdown products of collagen IV was observed at both 24 h and 4 wk after ischemic injury. Taken together, these findings indicate that the loss of endothelial cells following ischemic injury is not a major contributor to altered microvascular permeability, although renal microvascular endothelial cells are vulnerable to the initiation of apoptotic mechanisms following ischemic injury that can ultimately impact microvascular density.

endothelium; ischemia-reperfusion injury; kidney failure; apoptosis

ACUTE KIDNEY INJURY (AKI) is a relatively common clinical entity that is associated with considerable morbidity and mortality in humans (27, 28, 31, 39). Ischemia, due to hypotension or sepsis, is the most common cause of human AKI (26, 28), and recently there has been a renewed appreciation for the contribution that acute derangements in renal microvascular structure and function make toward the reduction in glomerular filtration rate, which is the functional hallmark of AKI (5, 20, 36). Endothelial cell injury resulting in endothelial cell swelling (18), altered endothelial cell-cell junctional contacts (38), and disruption of the endothelial cell monolayer (6) can have functional consequences for microvascular flow, permeability, and reactivity that ultimately have a negative impact on glomerular filtration rate.

In addition to the acute alterations in renal microvascular function that serve to initiate and extend injury in the early phases of AKI, there is growing evidence that chronic alterations in the renal microvasculature occur following the initial ischemic insult that may predispose survivors of an episode of AKI to the development of chronic kidney disease (CKD) (1, 8). In a rat model of AKI, microvascular rarefaction has been observed to occur several weeks after the inciting ischemic event (2). This loss in microvascular density has been demonstrated to be coincident with functional indicators of CKD, including diminished urinary concentrating ability and the development of progressive proteinuria. Furthermore, diminished microvascular reserve has been associated with persistent renal hypoxia (3), thus setting the stage for progression of CKD (23).

Although the acute loss of endothelial cell integrity and chronic microvascular rarefaction appear to have important pathophysiological consequences in ischemic AKI, the mechanisms involved in these alterations are not well defined. Angioregulatory proteins, such as vascular endothelial growth factor-A (VEGF-A), angiopoietin (Ang)-1, and Ang-2, are important mediators of endothelial cell and blood vessel survival, growth, regression, and function. In a rat model of ischemic AKI, VEGF protein levels have been demonstrated to be significantly elevated 24 h following the inciting ischemic insult (40, 41) and trending toward baseline at 72–96 h following ischemia (41). While changes in Ang protein levels have not been described in animal models of ischemic AKI, an increase in expression of the Ang receptor, Tie2, has been demonstrated to occur following acute ischemic insult in the rat kidney, with a peak expression at 48 h (41). The structural and functional consequences of changes in angioregulatory protein levels on endothelial cells and the renal microvasculature following acute ischemic injury are relatively unknown. Although apoptosis is a major contributor to the pathophysiology of AKI (10, 33), the focus has primarily been on tubular epithelial cells during the acute phase of AKI. Apoptosis has been described in the kidney interstitium following acute ischemic injury to the rat kidney (19); however, the cells involved were not extensively characterized and were thought to be structurally unrelated to the microvasculature. We hypothesized that endothelial cell loss from the renal microvas-
culture via apoptosis plays a significant role in the altered microvascular permeability and the microvascular rarefaction observed following acute ischemic injury to the kidney. Utilizing a mouse model of ischemic AKI, we demonstrate that endothelial cell loss is not the primary mechanism for the acute increase in microvascular permeability. In addition, we demonstrate that activation of caspase-3 in renal microvascular endothelial cells occurs and that the increase in caspase-3 activation is antecedent to microvascular rarefaction following ischemic injury.

MATERIALS AND METHODS

**Animals and experimental models.** Tie2-GFP mice (on an FVB/NJ background), which express green fluorescent protein (GFP) under the direction of the endothelium-specific receptor tyrosine kinase (Tie2) promoter (30), were obtained from Jackson Laboratory (Bar Harbor, ME) and as a kind gift from Dr. Mervin Yoder (Indiana University School of Medicine). FVB/NJ mice were obtained from Jackson Laboratory. All experiments were conducted in accordance with The Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996) and approved by the Institutional Animal Care and Use Committee. Male mice, weighing 20–25 g (10–12 wk), were anesthetized with 5% halothane for induction followed by buprenorphine HCl (0.01 mg/kg) subcutaneously and 1.5% halothane for maintenance and then placed on a homeothermic table to maintain core body temperature at 37°C. A midline incision was made, the renal pedicles were isolated, and bilateral renal ischemia was induced by clamping the renal pedicles for 20 min with microserrefines. The time of ischemia was chosen to obtain a reversibility of the clamps. Reperfusion time varied between 1.5% halothane for induction followed by buprenorphine HCl (0.01 mg/kg) subcutaneously and 1.5% halothane for maintenance and then placed on a homeothermic table to maintain core body temperature at 37°C. A midline incision was made, the renal pedicles were isolated, and bilateral renal ischemia was induced by clamping the renal pedicles for 20 min with microserrefines. The time of ischemia was chosen to obtain a reversible model of ischemic AKI without significant mortality over the ensuing 4 wk. After removal of the microserrefines, reperfusion was monitored visually before closure of the abdominal surgical wound. One milliliter of prewarmed (37°C) sterile saline was instilled into the peritoneum at the time of closing. Animals were allowed to recover on a homeothermic pad to maintain body temperature until the righting reflex was restored. For some experiments, a 30-min bilateral renal artery clamp was performed to induce more severe AKI. Sham surgery consisted of an identical procedure, with the exception of immediate release of the clamps. Reperfusion time varied between 24 h and 30 days. For experiments involving live multiphoton microscopic imaging of mouse kidneys, an intravenous jugular catheter was placed at the start of the image acquisition. A Bio-Rad IRIS SPOT Laser Scanning Confocal/Multiphoton Scanner (Hercules, CA) with an excitation wavelength of 800 nm for Hoechst was attached to a Nikon Diaphot inverted microscope (Fryer, Huntley, IL), as described by Dunn et al. (15).

**Animal Control.** Male mice, weighing 20–25 g (10–12 wk), were anesthetized with 5% halothane for induction followed by buprenorphine HCl (0.01 mg/kg) subcutaneously and 1.5% halothane for maintenance and then placed on a homeothermic table to maintain core body temperature at 37°C. A midline incision was made, the renal pedicles were isolated, and bilateral renal ischemia was induced by clamping the renal pedicles for 20 min with microserrefines. The time of ischemia was chosen to obtain a reversible model of ischemic AKI without significant mortality over the ensuing 4 wk. After removal of the microserrefines, reperfusion was monitored visually before closure of the abdominal surgical wound. One milliliter of prewarmed (37°C) sterile saline was instilled into the peritoneum at the time of closing. Animals were allowed to recover on a homeothermic pad to maintain body temperature until the righting reflex was restored. For some experiments, a 30-min bilateral renal artery clamp was performed to induce more severe AKI. Sham surgery consisted of an identical procedure, with the exception of immediate release of the clamps. Reperfusion time varied between 24 h and 30 days. For experiments involving live multiphoton microscopic imaging of mouse kidneys, an intravenous jugular catheter was placed at the start of the image acquisition. A Bio-Rad IRIS SPOT Laser Scanning Confocal/Multiphoton Scanner (Hercules, CA) with an excitation wavelength of 800 nm for Hoechst was attached to a Nikon Diaphot inverted microscope (Fryer, Huntley, IL), as described by Dunn et al. (15).

**Data analysis.** All results represent *n* = 3, unless noted otherwise. Results are expressed as means (SD). Band density data were analyzed for significance by unpaired Student’s *t*-test, and an *α* = 0.05 was utilized to determine significance. Microvascular rarefaction was analyzed for significance by a χ² test, and an *α* = 0.01 was utilized to determine significance.

**RESULTS**

**Model of acute renal failure.** Tie2-GFP mice (on an FVB/NJ background) and FVB/NJ mice that underwent bilateral clamping...
ing of the renal pedicles for 20 min developed AKI with a peak in serum creatinine levels at 1.4 mg/dl (SD 0.4) 24 h following ischemia compared with 0.1 mg/dl (SD 0.05) in sham-operated animals (Fig. 1). Serum creatinine concentrations in posts ischemic mice returned to baseline levels by 16 days following ischemia. Tie2-GFP mice and FVB/NJ mice that underwent bilateral renal clamping for 30 min developed significant AKI with a serum creatinine of 3.1 mg/dl (SD 0.5) at 24 h following ischemic injury, although mortality for this group exceeded 80% in the 72 h following ischemia.

**Integrity of the microvascular endothelial monolayer is intact in areas of increased microvascular permeability following ischemic injury.** Alteration of microvascular permeability has important pathophysiological implications for overall kidney function in AKI. To investigate the relationship between the integrity of the endothelial cell monolayer and altered microvascular permeability in this model of AKI, we infused Texas-Red-labeled albumin 24 h after ischemic injury and utilized intravital, multiphoton microscopy to reconstruct three-dimensional images of the microvasculature in areas of increased permeability. In all of the reconstructed volumes examined \((n = 3\) with \(7–8\) volumes reconstructed/mouse), we did not observe loss of endothelial cells in the areas of increased microvascular permeability following a 20- or 30-min bilateral renal artery clamp (Fig. 2).

**Microvascular rarefaction occurs in a mouse model of AKI.** To examine the chronic consequences of ischemic AKI on the microvascular density in mice, we quantified renal microvascular density 4 wk following an ischemic insult in our mouse model of AKI. We utilized an anti-cablin antibody to identify renal microvascular structures \((7)\) \((\text{Fig. 3, A and B})\). We found a significant 45% reduction in the microvascular density 4 wk after an ischemic insult in mice compared with age-matched, sham-operated control mice \((n = 4, \text{Fig. 3C})\).

**Caspase-3 is activated in renal microvascular endothelial cells following ischemic injury.** Given the degree of microvascular rarefaction observed in this model of ischemic AKI, we next sought to examine the extent of endothelial cell apoptosis. We examined nuclear morphology and TUNEL staining in the Tie2-GFP mouse at 1, 2, 8, 16, and 30 days following ischemia to quantify endothelial cell apoptosis. Although a significant number of tubular epithelial cells demonstrated evidence of apoptosis utilizing these methods at 1 and 2 days following ischemia (Fig. 4A), we did not observe endothelial cells that...
were TUNEL positive or had nuclear morphology consistent with apoptosis (fragmented, condensed nuclei) at any of the time points observed (n = 3, with 10–20 images observed per animal). To confirm the ability of these methods to detect endothelial cell apoptosis, we next injected an antibody capable of stimulating the Fas receptor and inducing apoptosis (21). TUNEL-positive endothelial cells were observed in the kidney (Fig. 4B) following injection of the Fas-stimulating antibody into Tie2-GFP mice, thereby confirming the ability of these methods to detect endothelial cell apoptosis. As an alternative marker for activation of the apoptotic pathway in renal microvascular endothelial cells following ischemic insult, we examined the localization of activated caspase-3 in this model of ischemic AKI. Activated caspase-3 was localized to endothelial cells in kidney tissue 1 day after ischemic insult (Fig. 4C), thus supporting the activation of apoptotic pathways in renal microvascular endothelial cells following ischemic insult. Of note, no positive immunostaining was observed in tissue from sham-operated control animals or in tissue from mice rendered ischemic and immunostained with secondary antibody alone (data not shown).

Expression of angioregulatory proteins is altered following ischemic injury. To evaluate the key regulatory proteins potentially influencing vessel survival and growth, we next quantified the expression levels of the angioregulatory proteins Ang-1 and Ang-2 following ischemic injury. Twenty-four hours after ischemic insult, we observed a significant increase in the protein expression of Ang-1 in the kidney to approximately eight times the level observed in sham-operated control animals [8.3 (SD 0.3) in ischemia vs. 1.0 (SD 0.8) in sham; Fig. 5A], but no significant difference in Ang-2 levels compared with sham-operated control animals [0.6 (SD 0.4) in ischemia vs. 1.0 (SD 0.2) in sham; Fig. 5B]. Similar to Vannay et al. (40), 24 h after ischemic insult, we observed a significant increase in a band at ~24 kDa that is consistent with mouse VEGF164 (data not shown). Thirty days after ischemic insult, when peritubular microvascular density is diminished, an almost twofold difference in Ang-1 persisted, but there is no significant difference in the protein levels of VEGF or Ang-2 from mice that experienced ischemic AKI compared with sham-operated control mice (Fig. 5).

Because the breakdown of collagen IV by the increased gelatinolytic activity that occurs after acute ischemic insult (4, 37) can generate fragments with antiangiogenic effects from the NC1 domain of the α-chains of collagen IV (9, 22), we sought to examine the liberation of fragments from the NC1 domains of the α-chains of collagen IV following ischemic injury. Twenty-four hours after ischemia, we observed a significant increase in fragments from the NC1 domain of the α-1 chain of collagen IV (~26 kDa) and the NC1 domain of the α-2 chain of collagen IV (~25 kDa), which correspond to the angioinhibitory proteins arresten and canstatin (Fig. 6A). A
significant increase in the fragment consistent with arresten is also observed at 30 days following ischemic insult, although a significant increase in the fragment consistent with canstatin is not observed (Fig. 6).

DISCUSSION

Alterations in the integrity of the endothelial monolayer have important implications for organ function. In the kidney, elegant studies by Brodsky et al. have demonstrated that acute alterations in endothelial cells, including loss of endothelial cell-cell contacts and frank denudation of endothelial cells, occur in the renal microvasculature following ischemia-reperfusion injury (6), thus providing a structural basis for altered endothelial barrier function and altered microvascular reactivity (6, 20). Our laboratory has previously demonstrated that an acute increase in renal microvascular permeability occurs following ischemia-reperfusion injury, and that this functional alteration is accompanied by a reduction in endothelial cell-cell junctions, as determined by vascular endothelial-cadherin immunostaining (38). In this present study, we demonstrate that the principal finding in areas of increased permeability is an intact endothelial monolayer. This finding provides further evidence that altered endothelial cell-cell contacts, resulting in increased intracellular gap formation, are a prominent mechanism for the enhanced microvascular permeability following ischemic injury. However, our analysis cannot exclude the possibility that severely damaged and nonfunctional peritubular capillaries devoid of endothelium and without blood flow eventually reestablish flow and contribute to altered permeability.

While endothelial cell loss may not be the primary mechanism for increased microvascular permeability in this model of AKI at the time points examined, it certainly does not diminish the potential role that endothelial cell detachment may play in the acute alteration of vascular reactivity or the chronic loss of microvasculature that is observed following ischemic injury. In the aforementioned study by Brodsky et al. (6), infusion of exogenous endothelial cells in a renal artery clamp model of AKI resulted in not only the implantation of these cells into the microvasculature, but improvement in microvascular tone and flow. In regards to the microvascular rarefaction following ischemic injury to the kidney, Basile et al. utilized a microfill technique to demonstrate that a 30–50% decrease in renal microvascular density occurs following an episode of ischemia in a rat model of ischemic AKI (2) and CD31 immunostaining to demonstrate a similar decrease in a mouse model of renal ischemia (personal communication, D. P. Basile). In this study,

Fig. 4. Apoptosis in renal microvascular endothelial cells following ischemia-reperfusion injury. Terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL), nuclear morphology, and caspase-3 activation were examined in fixed kidney sections following sham operation and renal artery clamping to evaluate the extent of renal microvascular endothelial cell apoptosis. All images were obtained by confocal microscopy. A: representative TUNEL staining of a kidney section from Tie2-GFP mouse 24 h after ischemic injury. Numerous TUNEL-positive epithelial cells (red) were observed at 24 h after ischemia, but TUNEL-positive endothelial cells were not observed (n = 3 per time point and 10–20 images per animal). GFP-expressing endothelial cells are green. 4′,6-Diamidino-2-phenylindole (DAPI) (blue) was used as a counterstain to label nuclei. B: TUNEL-positive endothelial cells (arrowhead) were observed in kidney tissue fixed from Tie2-GFP mice 2 h after the intravenous infusion of an anti-mouse CD95 agonistic monoclonal antibody. GFP-expressing endothelial cells are green, and TUNEL-positive cells are red (colocalization is white). DAPI (blue) was used as a counterstain to label nuclei. C: representative image of fixed kidney sections from an FVB/NJ mouse 24 h after ischemic injury demonstrating immunolocalization of activated caspase-3 (green and arrowheads) to peritubular endothelial cells. DAPI (blue) was used as a counterstain. Bar = 50 μm.
we demonstrate a similar reduction of microvascular density in the mouse following ischemic injury. We hypothesized that renal endothelial cell apoptosis was a key contributor to renal microvascular dropout following ischemic injury. Although we did not observe endothelial cell necrosis or either nuclear morphology or TUNEL staining consistent with endothelial cell apoptosis, we observed activation of caspase-3, a typical proapoptotic executioner protease, in endothelial cells (33). A prior study in a rat model of ischemic AKI by Forbes et al. (19) provided evidence of TUNEL-positive cells in the renal interstitium at 1, 4, 8, 16, 32, 64, and 180 days following the ischemic insult. However, they did not find evidence that these cells were localized to the vasculature. These findings lead them to conclude that there was no quantitative change in the apoptotic rate of vascular cells following ischemic injury. Although we anticipated that utilizing the Tie2-GFP mouse would improve our ability to detect positive TUNEL staining in endothelial cells, the fact that we did not identify TUNEL-positive endothelial cells is not entirely surprising. Given the long-time scale of microvascular rarefaction following ischemic insult and the rapid clearance of apoptotic cells, the overall prevalence of endothelial cell apoptosis and the potential for observing it by TUNEL is very low. In addition, although the TUNEL reaction has been shown to have excellent specificity for examining apoptosis of epithelial tubular cells in ischemic kidney tissue, the sensitivity was found to be approximately 61% (11). Even rapidly initiating apoptosis by the infusion of a stimulatory antibody to the FAS receptor in Tie2-GFP mice in our study did not result in a prominent number of TUNEL-positive endothelial cells.

Detection of caspase-3 activation in a renal artery clamp model of ischemic AKI has been reported to correlate with TUNEL staining in the renal tubules (41). While the detection of caspase-3 in kidney tissues following ischemic injury may be a sensitive marker of endothelial cell apoptosis, activated caspase-3 has been demonstrated to function as a mediator of cellular functions distinct from its role as an effector caspase in apoptosis (25, 35). Activated caspase-3 has been shown to play a role in cell proliferation, differentiation, and catabolism in a variety of cell types (13, 14, 16, 17, 29, 32, 43, 44). Potentially germane to our present study is a study demonstrating that activation of caspase-3 inhibits cell cycle progression of pe-
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ripheral B cells (42). Activation of a similar pathway in endothelial cells leading to diminished mitosis could provide an alternative explanation for microvascular rarefaction following ischemic injury.

Our examination of angioregulatory proteins that can influence vascular maintenance and survival in the kidney following ischemic injury revealed that there is a significant increase in the level of Ang-1 and a nonsignificant decrease in Ang-2 at 24 h following ischemic injury. The increase in Ang-1 corresponds to an increase in VEGF that has been observed previously (40, 41). Conventional views hold that, under the influence of VEGF, Ang-1 activation of Tie2 stabilizes vascular beds, while Ang-2 serves to block Ang-1 activation of Tie2 and destabilize vascular beds. Consequently, it would be anticipated that the acute changes in angioregulatory proteins we observed following ischemic injury in the kidney would serve to stabilize vascular beds and preserve microvascular density. However, a recent study has demonstrated that the Tie2 receptor is only transiently upregulated at 48 h following ischemic injury (41). Thus a mismatch between receptor and ligand expression occurs that could preclude conventional mechanisms of vascular maintenance and survival. This mismatch may also partially explain why an increase in renal microvascular permeability is observed 24 h following ischemic injury (38), as opposed to the conventional view that an increase in Ang-1 would diminish vascular permeability that would otherwise be accentuated by increasing VEGF levels. In addition, there is growing evidence that the interplay of these angioregulatory proteins is more complex than the conventional view, as exemplified by a recent study that demonstrates a protective effect of Ang-2 in stressed endothelial cells instead of a destabilizing effect (12). Alternatively, and potentially more likely, the increase in angiogenic factors observed after ischemic injury in the kidney may be offset by an increase in antiangiogenic factors. An increase in the antiangiogenic factor angiostatin following acute ischemic injury has been previously reported (4), and in our present study we provide evidence consistent with an increase in antiangiogenic factors liberated from the breakdown of collagen IV in the kidney following acute ischemic injury.

In summary, we have demonstrated that, although the endothelial cell monolayer is primarily intact in areas of increased renal microvascular permeability following ischemic injury, there is concurrent activation of proapoptotic proteases in endothelial cells, which heralds the ultimate microvascular rarefaction that occurs. This activation of proapoptotic proteases occurs in the setting of potentially competing influences of angiogenic and antiangiogenic factors. Despite the emerging complexity of the mechanisms regulating microvascular stability, continued elucidation of the mechanisms involved in renal microvascular alterations following ischemic injury should provide novel pharmacological approaches toward ameliorating the acute and chronic consequences of AKI.

ACKNOWLEDGMENTS

We thank Robert Bacallao for his kind gift of the antibody to cablin.

GRANTS

This study was supported by a National Kidney Foundation of Indiana grant and a Jackstädt ($134-10.003) grant to M. Hörbelt; and National Institute of Diabetes and Digestive and Kidney Diseases Grants (50621, 61594), Norman S. Coplon Extramural Research Grant of Satellite Healthcare, and a Kidney and Urology Foundation of America-American Society of Nephrology Research Grant to T. A. Sutton.

REFERENCES

18. Flores J, DiBona DR, Beck CH, Leaf A. Activation of a similar pathway in endothelial cells leading to diminished mitosis could provide an alternative explanation for microvascular rarefaction following ischemic injury.

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