Intravital videomicroscopy of peritubular capillaries in renal ischemia

TOKUNORI YAMAMOTO,1 TETSUHIRO TADA,2 SERGEY V. BRODSKY,3 HIROYOSHI TANAKA,1 EISEI NOIRI,4 FUMIHIKO KAJIYA,5 AND MICHAEL S. GOLIGORSKY3

1Departments of Urology and Medical Engineering, Kawasaki Medical School, Okayama 701-0114; 2Department of Electrical Engineering, Okayama University of Science, and 3Department of Cardiovascular Physiology, Okayama University School of Medicine and Dentistry, Okayama 700-0005; 4Department of Nephrology and Endocrinology, The University of Tokyo, Tokyo 113-8655, Japan; and 3Departments of Medicine, Physiology, and Biophysics and Program in Bioengineering, State University of New York at Stony Brook, Stony Brook, New York 11794-8152

Received 3 October 2001; accepted in final form 14 January 2002

Yamamoto, Tokunori, Tetsuhiro Tada, Sergey V. Brodsky, Hiroyoshi Tanaka, Eisei Noiri, Fumihiko Kajiya, and Michael S. Goligorsky. Intravital videomicroscopy of peritubular capillaries in renal ischemia. Am J Physiol Renal Physiol 282: F1150–F1155, 2002. First published January 29, 2002; 10.1152/ajprenal.00310.2001.—The recent refinement and computerization of intravital microscopy have permitted us to monitor microcirculation in vivo with minimal invasion. Here, we report on the first findings made with the use of a pencil-lens intravital microscope as applied to the ischemic rat kidney. Peritubular capillary and glomerular blood flow were monitored under basal conditions, during renal artery occlusion, and immediately after release of the clamp. Erythrocyte velocity was calculated as an angle in consecutive spatiotemporal images. Intravital videomicroscopy during the reperfusion period showed intermittent cessation and partial recovery of blood flow in both peritubular and glomerular capillaries. Blood flow was uniformly orthograde under control conditions; however, the retrograde flow occurred on reperfusion. The patency of peritubular capillaries was partially compromised during the early reperfusion period but rapidly recovered. The recovery of glomerular microcirculation occurred faster than that of peritubular capillaries. We suggest that a functional vasculopathy develops very early in the course of ischemia-reperfusion in superficial cortical microvasculature and is more pronounced in peritubular capillaries, thus accounting for the development of patchy injury of tubular epithelia.

renal ischemia-reperfusion; glomerular capillaries

IN HUMANS AND EXPERIMENTAL animals with acute renal ischemia, despite a drop in the glomerular filtration rate to ~5% of the baseline level, morphological analysis of glomeruli and microvasculature yields no discernible pathological changes (5, 8, 14, 17, 21). The established morphological manifestations for this syndrome consist of patchy necrosis of tubular epithelial cells, their desquamation, and obstruction of the tubular lumen (15, 20).

Notwithstanding the apparent lack of morphological signs of vascular injury, investigators have repeatedly disclosed functional abnormalities of renal circulation in acute renal ischemia. Flores et al. (15) demonstrated that endothelial cells undergo early swelling, resulting in the narrowing of the lumen. This observation has been conceptualized as the “no-reflow” hypothesis (10, 23). A finding of the profound loss of acetylcholine-induced vasorelaxation in ischemic renal vasculature (11) supported this hypothesis. Conger et al. (3, 4) have demonstrated that vasorelaxation in response to stimuli generating endothelium-derived relaxing factor was inhibited in acute renal failure, and our laboratory has previously shown that nitric oxide (NO) production in response to bradykinin was suppressed in ischemic kidneys (12). Overexpression of adhesion molecules like intracellular adhesion molecule-1 (ICAM-1) by the vascular endothelium of the ischemic kidney has been demonstrated to play a major pathophysiological role in the development of renal dysfunction, and neutralizing anti-ICAM-1 antibodies significantly improved the outcome of renal ischemia (9). Furthermore, our laboratory has previously demonstrated that the endo-
the epithelium of renal microvessels in ischemic kidneys shows a loss of polarity in the expression of Arg-Gly-Asp-binding integrins, similar to that seen in the tubular epithelium (18). Collectively, these functional abnormalities strongly suggest that renal ischemia represents “vasomotor nephropathy,” resulting in a severe, lasting compromise of circulation. A lingering question that remains unanswered is, If a functional vasculopathy of the no-reflow type develops at the level of peritubular capillaries, could the resulting perpetuation of defective blood supply be responsible for the observed damage to the tubular epithelium?

Until recently, renal hemodynamics was studied using mostly clearance techniques, dye-dilution methods, entrapment of microcapsules or fluorescent indicators, the Doppler effect, or micropuncture techniques. These methods, although they have led to significant discoveries, pose limitations in discerning in vivo microcirculatory patterns, especially at the level of individual peritubular capillaries. Steinhausen and co-workers (22) provided an early unique description of videomicroscopic findings during recovery from acute renal ischemia, but no data are available on early postischemic microcirculation. The recent refinement of intravital videomicroscopy, combined with sophisticated image analysis, has permitted us to monitor microcirculation in vivo with minimal invasion (25, 26). Here, we report on the first findings made with the use of pencil-lens intravital microscopy as applied to the ischemic rat kidney. These findings strongly support the hypothesis that vasomotor nephropathy does develop in the ischemic model of acute renal failure and that the compromise to the renal microcirculatory blood flow significantly outlasts the duration of renal artery clamping.

METHODS

Surgical procedure. All experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and approved by the institutional Animal Care and Use Committee. Male Wistar-Kyoto rats weighing 160–180 g were allowed food and water ad libitum. After an overnight fast, animals were anesthetized with 100 mg/100 g of Inactin. The animals were placed on a heated surgical table, rectal temperature was maintained at 37°C, and a tracheostomy was performed. A subcutaneous injection of 250 U/kg heparin was given 15 min before the operation. A 2.5-cm midilaparotomy was performed, the left kidney was exposed, decapsulated, and immobilized in a Lucite kidney cup, and the renal artery was separated from the renal vein. The kidney was covered with mineral oil and kept continuously irrigated with normal saline. Renal ischemia was initiated by clamping the left renal artery with microserrifines (Fine Science Tools, Forster City, CA). After 45 min, the clamp was removed and the monitoring of blood flow was resumed, as detailed below. Throughout the entire experiment, the surface of the kidney was protected from drying, as detailed immediately above. When recordings of blood flow were performed 24 h postoperatively, animals were anesthetized with a combination of ketamine hydrochloride (6.0 mg/100 g) and xylazine hydrochloride (0.77 mg/100 g), the wound was closed, and the animals were used for videomicroscopy studies 24 h later that employed the above protocol.

Intravital microscopy and analysis of peritubular blood flow. Experiments were performed in animals surgically prepared as described above. Renal cortical peritubular capillaries were visualized with a pencil-lens probe, charge-coupled device videomicroscope with a tip diameter of 1 mm, as previously detailed (26). The probe had a magnification of ×520, depth of field <60 μm, and spatial resolution of 0.86 μm, permitting identification of individual erythrocytes (25, 26). Illumination was provided by a concentric set of optical fibers transmitting light from a xenon AC 100-V light source. Video signals were digitized with an analog-to-digital converter and fed into a digital videocassette recorder (DVCAM, Sony) interfaced with a computer. Analysis was performed using the NIH Image program combined with Matlab or specifically written programs. Peritubular capillary blood flow was recorded with a pencil-lens microscope brought into direct contact with the decapsulated renal surface, which had been covered with mineral oil to prevent evaporation. In studies of glomerular microcirculation, a superficial slice through the renal cortex was made to ensure microscope access to glomeruli. Images were recorded on digital videocassette tapes before and after renal artery clamping. After 45-min renal artery occlusion, the clamp was removed and flow was initiated. The consecutive images of blood flow were collected at a rate of 30 frames/s for 60 min postoperatively. Images were analyzed using the freeze-frame mode. The velocity of red blood cells (RBC) in individual segments of the peritubular capillaries was analyzed using a specifically designed adaptation of the previously developed algorithm (13). Specifically, a line segment was set along a capillary bed in sequentially videotaped images, and a spatiotemporal image was constructed (the line-shift method), allowing us to discern differences in gray level during the passage of RBC. The angle of a line-shift striped pattern was estimated to compute the erythrocyte velocity vector.

RESULTS

RBC velocity in peritubular capillaries after renal ischemia. RBC velocity in peritubular capillaries averaged 1,069 ± 146 μm/s (n = 15). As expected, clamping the renal artery resulted in a complete cessation of blood flow accompanied by blanching of the surface and visualization of individual RBC halted within the vessels (Figs. 1 and 2; supplementary video) and showed partial recovery by 15–20 min (Fig. 1, frame 65′, and supplementary video). Twenty-four hours posts ischemia, peritubular capillary blood flow remained significantly diminished, representing only one-quarter of the control level (227 ± 113 μm/s).

Direction of flow in peritubular capillaries after renal ischemia. Analysis of videotapes and calculations of RBC velocity posts ischemia revealed that the normally orthograde direction of RBC movement was interrupted in the posts ischemic period with the appearance of several patterns. In some capillaries, the flow was switching from the orthograde to the retrograde; in
other capillaries, RBCs displayed a pulsatile behavior with periodic stagnation followed by the resumption of flow in the orthograde direction (Fig. 3; supplementary video illustrates the observed patterns of blood flow in the peritubular capillaries).

Functional and nonfunctional peritubular capillaries. An additional feature characteristic of postischemic microcirculation in the peritubular capillaries is represented by their patency. As shown in Fig. 2, blood flow in some capillaries was abated or stagnant. To characterize the dynamics of this process, we analyzed stacks of consecutive images and calculated the number of nonfunctioning capillaries at different time points after the release of the renal artery occlusion (the number of functioning capillaries before the occlusion was expressed as 100%). The data presented in the inset to Fig. 2 summarize this analysis. They show the remarkable functional plasticity of peritubular capillaries, which underwent a rapid opening on initiation of the reperfusion, immediately followed by a near-complete closure. The patency of the visible peritubular capillaries was recovered by 60 min.

Vasomotion of interlobular arteries postischemia. Analysis of stacked images before and after the release of the renal artery clamp allowed us to monitor the diameter of individual interlobular arteries in real time. (Notably, this type of analysis could not be performed technically on capillary vessels because of their low contrast.) The results were obtained in five differ-
ent preparations. The major finding in all cases was that postischemic interlobular arteries showed a trend toward vasodilation, compared with the baseline diameter (129 ± 14% increase, n = 5, P > 0.05). Thus the observed changes in blood flow in peritubular capillaries, at least immediately after removal of the clamp, could not be attributable to vasoconstriction in upstream resistance arteries. Therefore, we next analyzed preglomerular and glomerular blood flow.

Glomerular hemodynamics after renal ischemia. Minimally invasive (cortical surface cut) videomicroscopy of glomerular circulation was performed before clamping of the renal artery and after release of the clamp. Figure 4 provides representative consecutive images of a glomerulus and summarizes the dynamics of microcirculation. On release of the clamp, there was an almost instantaneous recovery of glomerular blood flow. This, however, was followed within 1–2 min by an oscillating pattern of cessation and partial recovery of blood flow (see supplementary video, which illustrates the observed oscillating pattern of glomerular capillary blood flow). During the periods of abated glomerular microcirculation, the shunting of blood from the afferent to the efferent arterioles inside glomeruli, bypassing the bulk of glomerular capillaries, was occasionally observed (not shown).

DISCUSSION

The results of minimally invasive videomicroscopy of peritubular capillary blood flow, summarized herein, provide direct confirmation of a view that acute renal ischemia represents vasomotor nephropathy. This hypothesis was based on studies performed in Leaf's laboratory (6) demonstrating endothelial cell swelling on initiation of reperfusion of a previously ischemic organ. These observations were conceptualized as a no-reflow state of postischemic hemodynamics (10, 23). Although never proven directly, this hypothesis received substantial circumstantial support (3, 4, 9, 11, 12, 18). Using intravital videomicroscopy of renal microcirculation during the immediate reperfusion period, we were able to provide a set of novel findings directly demonstrating that 1) erythrocyte velocity in peritubular capillaries exhibited an immediate partial recovery after release of renal artery occlusion, followed by a profound and sustained deceleration of the blood flow; 2) a proportion of capillaries temporarily lost their patency; and 3) motion analysis of blood flow in postischemic capillaries showed shifts from the orthograde to the retrograde direction. Each of these patterns separately, and especially their simultaneous coexistence in the postischemic kidney, directly explain the mechanics of the no-reflow phenomenon. The observed changes in the direction of blood flow or its stagnation undoubtedly contribute to the defective per-
fusion of peritubular capillaries and poor oxygen transport to proximal tubular epithelial cells.

Disturbances in renal circulation after renal artery occlusion have been the focus of many investigations. Studies with tracer washout techniques have produced evidence for preferential reduction in the cortical blood flow in dogs during hemorrhagic hypotension (2). One-hour renal artery occlusion has been shown to cause a primary disturbance in postglomerular perfusion (as studied with fluorescently labeled globulins), thus leading to the damage of tubular epithelia (24). On the other hand, micropuncture studies of ischemic kidneys showed proportional increases in afferent and efferent arteriolar resistance, resulting in a preserved filtration fraction and a fall in glomerular blood flow (5). Using single-fiber laser Doppler flowmetry, Hellberg et al. (7) demonstrated trapping of RBC in the outer medulla, causing the shunting of blood to the inner medulla. Thus diverse technical approaches provided vastly different characteristics of postischemic circulation, thus incriminating different segments of the renal vasculature in the development of no-reflow. The data presented above, although limited to the superficial cortical vasculature, suggest that functional vasculopathy develops early in the course of reperfusion and simultaneity in different vascular beds, as discussed below.

To the best of our knowledge, the earliest videomicroscopic study of peritubular blood flow after ischemia was performed by Steinhansen et al. (22). Analyzing RBC velocity in peritubular capillaries 3 days postischemia, these investigators demonstrated that it was decreased to one-quarter of control values. Unfortunately, no data on the immediate reperfusion period were obtained in that study. The present work, based on the use of pencil-lens videomicroscopy, continues this line of investigations, focusing on immediate postocclusive microcirculation in peritubular capillaries, the site responsible for oxygen transport to the proximal tubular epithelium. The apparent lack of a detectable early vasoconstriction of intralobular arteries suggests that the impedance to peritubular blood flow occurs downstream, i.e., at the level of the preglomerular or postglomerular vasculature. Indeed, glomerular microcirculation shows a pattern similar to peritubular capillary flow: initial recovery of blood flow is followed by its temporal cessation, despite the fact that renal artery occlusion is no longer present. The rate of postischemic recovery of microcirculation in these capillary beds, however, is different. Although glomerular circulation recovers by 60 min, peritubular capillaries show a significant delay in the recovery of blood flow (Fig. 5). This finding suggests that peritubular capillaries are more vulnerable to acute ischemia or that the rate of their functional recovery is hampered by the effects exerted from the ischemic epithelial cells.

The molecular mechanism(s) responsible for perturbed vasomotion, although of great importance, was beyond the scope of this study. However, the available data suggest that fluid shear stress to endothelial cells represents a physiological stimulus for eNOS activation (16, 19). Stagnated blood flow, therefore, should necessarily result in suppressed NO production. The normal pattern of shear-stimulated eNOS could be further aggravated during reperfusion as a result of the observed shifts from the orthograde to the retrograde blood flow. Moreover, the concomitant generation of reactive oxygen species may further deplete bioavailable NO through the generation of peroxynitrite (12). Our initial attempts to correct renal hemodynamics by transplanting endothelial cells are summarized in a companion study (1). Further studies will be focused on elucidation of mechanisms responsible for the observed hemodynamic disturbances. The use of intravital microscopy opens the perspective for direct analysis of pharmacological interventions, which could correct this abnormality.

These studies were supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-45462, DK-54602, and DK-52783 (M. S. Goligorsky) and American Heart Association Fellowship 0120200T (S. V. Brodsky).

Supplementary digital video files for Figs. 1, 3, and 4 are available at http://ajprenal.physiology.org/cgi/content/full/282/6/F1150/DC1.

REFERENCES


13. Ogasawara Y, Takehara K, Yamamoto T, Hashimoto R, Nakamoto H, and Kajiya F. Quantitative blood velocity map-


