Kidney Tubular Cell Regeneration Starts in the Deep Cortex after Ischemia

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Purpose: In kidneys exposed to ischemia/reperfusion (I/R), the periodic and regional changes of loss and restoration of tubular epithelial cells and the influence of these processes for renal function remain to be defined. We investigated the loss and regeneration of tubular cells in each nephron segment at various times after I/R.

Methods: Mice were subjected to 30 min of bilateral renal ischemia and were administered 5-bromo-2’-deoxyuridine (BrdU) 20 hours before harvest kidneys. The numbers of tubular cell nuclei, BrdU-incorporating cells and proliferative cell nuclear antigen (PCNA)-positive cells were analyzed by PAS-staining and immunohistochemistry.

Results: Thirty minutes of ischemia induced loss of tubular epithelial cells in the outer stripe of the outer medulla. The loss of tubular epithelial cells peaked 24 hours after ischemia. After the maximum decrease, recovery of number of tubular epithelial cells was observed from 3 days after I/R in the outer medulla and from 5 days in the cortex. The tubular cell numbers were inversely correlated with the changes in concentrations of plasma creatinine and BUN by Pearson correlation, indicating that the decrease and increase of tubular epithelial cell numbers reflect functional failure and recovery, respectively. Cell proliferation as determined by BrdU-incorporating appeared in the deep cortex from 3 days after ischemia.

Conclusion: The recovery of renal function was found to significantly correlate with the restoration of tubular cells. Furthermore, the regeneration of tubular cells started in the tubules of the deep cortex, suggesting that it may be a great proliferative cell niche.

Key Words: Ischemia, Cell proliferation, Regeneration, Bromodeoxyuridine, Proliferating cell nuclear antigen

INTRODUCTION

In the kidney, ischemia/reperfusion (I/R) results in apoptotic and necrotic death of tubular epithelial cells, impairs renal function, and commonly causes acute renal failure (ARF)¹,². Nephron segments of the kidneys possess a remarkable different susceptibility to I/R. The S3 segment of the outer medulla is most susceptible to I/R injury. After severe damage, the kidney can recover from the dysfunction and cell loss within several days of blood flow recovery, although several recent studies have demonstrated that the kidneys undergo mild permanent changes, such as expansion of the interstitial space, depending on damage severity³–⁶. Post-ischemic restoration of tubular
epithelial cells has accounted for the fact that the cells surviving the injury divide, dedifferentiate, and finally mature into functional epithelial cells, although recently several investigators have reported that bone marrow–derived extrarenal cells contribute to the restoration of damaged tubular epithelial cells. However, the interrelationship in the loss and proliferation of tubular epithelial cells and the change of renal function after I/R remains to be clearly elucidated. Moreover, the profile of the regional restoration of tubular epithelial cells after I/R injury is unclear. In the present study, we evaluated 1) changes of renal function associated with loss and mitosis of epithelial cells, and 2) the profile of regional proliferation of tubular epithelial cells in post–ischemic kidneys. We found that the number of tubular epithelial cells reflected the renal function and that the proliferation of tubular epithelial cells started in the deep cortex and extended to other areas of the kidney.

MATERIALS AND METHODS

1. Animal Preparation

Experiments were performed in BALB/c male mice purchased from the Dae–Han Experimental Animal Center (Daejeon, Korea). In all cases, studies were conducted according to the animal experimentation guidelines issued by the Animal Care and Use Committee at Kyungpook National University. Animals were anesthetized with pentobarbital sodium (60 mg/kg body weight, BW; intraperitoneally, i.p.) prior to surgery. Body temperatures were maintained at 36.5–37.5°C throughout the procedure. Kidney ischemia was induced as previously described. For the 5-bromo-2-deoxyuridine (BrdU; Sigma, Saint Louis, MO)–incorporation experiment, mice were administered 50 mg/kg BW of BrdU i.p. 20 hours before harvest of kidneys.

2. Renal functional parameters

To evaluate concentrations of plasma creatinine (PCR) and blood urea nitrogen (BUN), blood were taken from the orbital sinus at 1, 4, 8, 12, 18, and 24 hours and 2, 3, 4, 5, 7, 9, and 16 days after either ischemia or sham–operation (n=4–7 per time point, total n=96). PCR concentration was measured using Beckman Creatinine Analyzer II (Beckman, Brea, CA), and BUN concentration was measured using BUN kit (Asan Pharm, Seoul, Korea).

3. Immunohistochemistry

Kidney fixation was accomplished as described previously. Kidney sections were stained through periodic acid Schiff (PAS) and immunohistochemistry. Immunohistochemical staining was performed as described below using the following antibodies: anti–BrdU (Serotec, Oxford, UK), anti–proliferative cell nuclear antigen (PCNA; DAKO, Copenhagen, Denmark), anti–Na/K–ATPase (Sigma; Saint Louis, MO), anti–aquaporin–1 (AQP1; Alomone Lab, Jerusalem, Israel), and anti–AQP2 (Alomone Lab, Jerusalem, Israel). To detect antigens, sections were deparaffinized with xylene and then rehydrated with 100, 95, and 80% ethanol. To unmask the antigen epitopes, slides were boiled in 10 mM sodium citrate buffer (pH 6.0) for 5 min, cooled to room temperature for 20 min, and then washed with PBS (2×5 min). To block the activation of endogenous peroxidase, slides were treated with 3% hydrogen peroxide (H₂O₂) in methanol for 30 min at 4°C and then washed with PBS (2×5 min). The sections were blocked with blocking buffer containing 1% bovine serum albumin (BSA) for 30 min at room temperature, incubated with primary antibodies in 1% BSA overnight at 4°C, washed with PBS three times, and then incubated with the respective biotinylated secondary antibodies for 60 min at room temperature. The sections were treated with ABC reagent (Vector Labora-