ORIGINAL INVESTIGATIONS

Pathogenesis and Treatment of Kidney Disease and Hypertension

Increased Endothelin 1 Expression in Adult-Onset Minimal Change Nephropathy With Acute Renal Failure

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• ***Background:*** **Acute renal failure (ARF) occurs in some adult patients with minimal change nephropathy (MCN). To investigate clinical and pathological factors associated with developing ARF, we compared clinical features and kidney pathological characteristics of endothelin 1 (ET-1) expression in patients with adult-onset MCN with and without ARF.** ***Methods:*** **The patient population consisted of 53 patients consecutively diagnosed with adult-onset MCN during a 10-year period. Based on creatinine clearance, 25 patients were assigned to the ARF group and 28 patients were assigned to the non-ARF group.** ***Results:*** **Clinical data show that the ARF group had a higher blood pressure, higher serum cholesterol level, and lower serum albumin level than the non-ARF group. Pathological data showed more severe foot-process effacement, interstitial edema, and flattened tubular epithelium in the same group. Greater ET-1 expression was detected in vessels, tubules, and glomeruli of the ARF compared with non-ARF group. The ARF group experienced a lower steroid response rate. However, there was no significant difference in stability of remission to steroid treatment in patients who achieved a remission.** ***Conclusion:*** **ARF associated with enhanced kidney ET-1 expression is a reversible complication of MCN that occurs frequently in patients with apparently expanded extracellular fluid. Presumptively, ARF may develop as an amplification of the underlying pathogenesis of MCN involved in enhanced ET-1 expression, which may be superimposed by a transient episode of circulatory insufficiency during diuretic treatment.** ***Am J Kidney Dis*** **45:818-825.**

INDEX WORDS: Endothelin 1 (ET-1); minimal change nephropathy (MCN); acute renal failure (ARF).

ACUTE RENAL FAILURE (ARF) has been associated with nephrotic syndrome caused by minimal change nephropathy (MCN).[1-9] Usually the deterioration in renal function is mild or moderate and reversible, although the renal failure may be irreversible and hemodialysis is required on rare occasions.[6] The pathogenesis of ARF in patients with MCN remains uncertain; however, it has been attributed to decreased intravascular volume, tubular obstruction secondary to renal interstitial edema, decreased ultrafiltration coefficient, or acute tubular necrosis.[1,3,7-9] Aggressive diuresis in combination with the use of angiotensin II–converting enzyme inhibitors or peritonitis also could be a precipitating factor for ARF in patients with nephrotic syndrome.[10,11] Among the proposed causes, low renal perfusion secondary to systemic volume depletion is the most persuading presumption regarding the pathological findings of acute tubular necrosis that have been reported in the past.[9] However, it usually occurs in nephrotic patients with heavier proteinuria, more severe hypoalbuminemia, and apparently expanded extracellular fluid.[1,7] In our clinical experience, renal failure usually is associated with expanded extracellular fluid, responding well to steroid and aggressive diuretic treatment. We explore pathological characteristics in these patients.

Endothelin 1 (ET-1), a peptide of 21 amino-acid residues isolated from porcine aortic endothelial cells, is the most potent vasoconstrictor known.[12] ET-1 acts on the renal vasculature, inducing a decline in blood flow and consequent reduction in glomerular filtration rate.[13] Many

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previous reports showed that ET-1 has a pivotal role in the pathogenesis of ARF of various causes, including ischemia, contrast medium, glycerol injection, and obstruction.[14-17] In addition, ET-1 has been found to be involved in the pathogenesis of MCN that has enhanced urinary excretion of ET-1.[18] There is evidence of increased immunostaining in steroid-resistant minimal change disease and focal segmental glomerulosclerosis.[19] In the present study, we attempt to explore the role of ET-1 in the pathogenesis of ARF in patients with MCN. In a retrospective study from April 1992 to December 2003, we collected all adult nephrotic patients with pathological diagnoses of MCN, with and without ARF, who were admitted to the hospital and analyzed their clinical features and pathological findings in the kidneys, especially for ET-1 expression and distribution.

METHODS

Patients

Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan) was established to serve as both a community hospital and referral medical center. A renal biopsy is recommended for all adult nephrotic patients who visit our outpatient clinics. Patients are able to refuse this procedure. From April 1992 to December 2003, in 60 of 212 adult patients who underwent renal biopsy on account of nephrotic syndrome, MCN was diagnosed on the pathological examination. Based on data for creatinine clearance measurements during a 2-month period from the renal biopsy, 28 patients with a decline in creatinine clearance at the time of biopsy of less than 25% compared with the best values during this period were selected as the non-ARF group, whereas 25 patients with a more than 35% decline in creatinine clearance were allocated to the ARF group. Because of difficulty determining whether there was renal failure, 7 patients with creatinine clearance changes between 25% and 35% were excluded. Creatinine clearance was calculated by dividing the amount of creatinine in a 24-hour urine sample by the concentration of creatinine in blood.

All patients had a detailed history taken and underwent a thorough physical examination to exclude other causes of ARF, including use of nonsteroidal anti-inflammatory drugs or other potentially renal toxic agents, the presence of renal vein thrombosis, and renal hypoperfusion caused by sepsis or dehydration. None of the selected patients was excluded for the reasons listed, and all patients were edematous. Two patients in each group had been administered an angiotensin II–converting enzyme inhibitor at the time of biopsy; for 2 patients in the ARF group, the treatment was initiated long before the appearance of impaired renal function. Arterial blood pressure was measured manually by using a sphygmomanometer. An inflatable cuff was placed around the upper arm at approximately the same vertical height as the heart. While listening with a stethoscope to the brachial artery at the elbow, the cuff was inflated until the artery was completely occluded, then pressure in the cuff was released slowly. Systolic blood pressure was obtained at the beginning of the first Korotkoff sound, whereas diastolic pressure was obtained at the end of the fifth Korotkoff sound. Fifty-three patients were enrolled in the study. Demographic and biochemical data were collected at the time of biopsy and every 2 to 4 weeks for the duration of the study.

Routine Pathological Examination

Renal biopsy specimens from all enrolled patients were obtained and prepared using standard procedures for light microscopy and electron microscopy. Two experienced pathologists who were blinded to patients' clinical profiles examined each pathological section. To analyze the extent of interstitial edema and flattened tubular epithelium, 4-μm thick sections stained with hematoxylin-eosin and periodic acid–Schiff were viewed at original magnification $\times$150 under a light microscope. We examined 3 sections/biopsy specimen, which contained 6 to 20 glomeruli. Degree of interstitial edema was graded according to the percentage of the area showing interstitial edema, as follows: 0 indicates absent; 1, less than 25%; 2, 25% to 50%; and 3, more than 50%. If the average score of 6 readings for each biopsy was greater than 1.5, interstitial edema was considered positive. Flattened tubular epithelium was assessed according to the percentage of area showing cuboid epithelium, loss of brush border, and greater lumen to epithelium height ratio by periodic acid–Schiff stain, as follows: 0 indicates absent; 1, less than 25%; 2, 25% to 50%; and 3, more than 50%. If the average score of 6 readings for each biopsy was greater than 1.5, flattened tubular epithelium was considered positive.

To assess the extent of foot-process effacement, ultrathin sections were prepared by routine processing, then observed at original magnification $\times$12,000 under a Jeol 1210 transmission electron microscope (Joel Ltd, Tokyo, Japan). One to 3 glomeruli were investigated in each section. Foot-process effacement was assessed according to the percentage of basement membrane with foot-process effacement, as follows: 0 indicates absent; 1, less than 25%; 2, 25% to 75%; and 3, more than 75%. The average score of all readings was calculated and used for analysis.

Immunohistochemistry

In addition to immunohistochemical study, renal tissues from 6 patients whose kidneys were removed for tumor growth were used as normal controls. To analyze the distribution and intensity of ET-1 expression, we used an antihuman ET-1 antiserum raised in mouse (Ab2786; Abcam Limited, Cambridgeshire, UK). This antibody shows little cross-reactivity to ET-2 and ET-3.[20] Paraffin-embedded sections were dewaxed by using xylene, and sections were rehydrated in a graded ethanol series. Their endogenous peroxidase was inactivated with 3% hydrogen peroxide in methanol for 10 minutes. Sections then were reacted with avidin and biotin to block the endogenous binding sites before incubation with mouse monoclonal antibodies to ET-1 at 1:250 dilution according to the manufacturer's instructions. Sections then were reacted with a biotinylated