Renal Tubular Apoptosis in Twin-to-Twin Transfusion Syndrome

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ABSTRACT

Twin-to-twin transfusion syndrome (TTTS) is caused by uneven shunting of blood between monochorionic twins, resulting in polycythemia in the recipient twin and growth restriction, anemia, and oliguria in the donor twin. Recent reports have described loss of proximal convoluted tubules in the kidneys of TTTS donor twins. In order to elucidate the pathogenesis of tubular deficiency in TTTS, we have reviewed the renal pathology in 25 twin pairs with autopsy-proven TTTS. Loss of differentiated proximal tubules, associated with atrophy of medullary tubules, was identified in 12/25 donor twins. In seven of these cases (all > 23-wk gestational age), the kidneys showed diffuse or partial tubular atrophy without evidence of cell death, similar to previously reported patterns. In five cases (all between 18- and 22-wk gestation), proximal and medullary tubules showed active injury characterized by markedly increased apoptosis, cell detachment, and intraluminal cell debris associated with calcifications. Tubular apoptosis tended to be more prevalent in donor fetuses with greater inter-twin body weight discordance, consistent with a more severe degree of TTTS. These results extend the spectrum of tubular alterations in TTTS to include an early stage of active apoptotic injury. The temporal distribution of injury patterns suggests that apoptotic injury of proximal and medullary tubules may be a precursor to partial or diffuse tubular atrophy. We speculate that the risk for development of tubular apoptosis in TTTS depends on the severity and timing of the hemodynamic imbalance, whereby early mid-trimester fetuses may be more vulnerable.

Key words: kidney, monochorionic twin, programmed cell death, renal tubular dysgenesis, terminal deoxynucleotidyl transferase-mediated dUTP-fluorescein isothiocyanate nick-end labeling

INTRODUCTION

Approximately one in five diamniotic-monochorionic twin pregnancies is complicated by a hemodynamic imbalance known as the chronic twin-to-twin transfusion syndrome (TTTS) [1,2]. This syndrome is characterized by shunting of blood through placental vascular communications from the donor twin to the recipient twin [3,4]. As a result of this unbalanced fetofetal transfusion, the smaller and growth-restricted donor twin becomes hypovolemic, anemic, and oliguric, and develops oligohydramnios. The recipient twin becomes hypervolemic, polyuric, and polycythemic, and develops polyhydramnios [1,5]. Both twins are at risk of developing hydrops fetalis and, if untreated, the condition carries a 80–100% mortality.

Autopsy studies of donor and recipient twins have described inter-twin differences in body and
organ weight, whereby a discordant heart/body weight ratio is generally considered the pathological hallmark of severe chronic TTTS [1,5]. In addition, chronic TTTS has been associated with characteristic alterations involving the proximal convoluted tubules (PCT) of the donor kidney. While “decreased mass of tubular tissue” [6] and “degenerative changes in the tubules” [7] of the donor kidney were recognized more than four decades ago, these renal alterations have been the focus of renewed interest in the past few years [8–10].

The pathogenesis of tubular injury in TTTS donors remains poorly understood. In view of the apparent absence of degenerative changes or cell death, the PCT loss in donor kidneys has been attributed to a mid-gestation developmental defect secondary to hypoperfusion [8,10]. Accordingly, the tubular alterations in TTTS have been termed “renal tubular dysgenesis” [8,10], analogous to the well-described hereditary or acquired renal condition characterized by PCT deficiency [11,12].

In order to further elucidate the pathogenesis of TTTS-associated tubular alterations, we have reviewed the renal findings in a large series of twins with autopsy-proven chronic TTTS, culled from the autopsy files of Women and Infants Hospital of Rhode Island. Our findings extend the spectrum of tubular lesions in TTTS to include an active phase, characterized by tubular apoptosis, that precedes the previously described stages of advanced tubular atrophy. Increased knowledge of the renal pathophysiology of TTTS may lead to the development of improved pre- or postnatal management strategies for this often lethal condition.

METHODS

Patient selection

The autopsy records of Women and Infants Hospital were searched for cases with an autopsy-confirmed diagnosis of chronic TTTS. The following inclusion criteria were used: 1) both donor and recipient twins were autopsied, and autopsy records, slides and/or paraffin blocks were available for both twins; 2) the existence of diamniotic-monochorionic placentation was confirmed by pathological analysis; and 3) the heart/body weight ratio of the recipient twin was at least 25% greater than that of the donor twin, consistent with severe chronic TTTS. The autopsy records were reviewed and body and kidney weights recorded.

Analysis of renal and tubular morphology

Hematoxylin and eosin-stained kidney sections from donor and recipient twins were reviewed and compared with age-matched control kidneys from singleton fetuses without congenital anomalies. The slides were reviewed for the presence and patterns of tubular alterations. The PCT density and the number of glomerular generations were determined. To determine the PCT density, the number of recognizable PCT profiles was determined in at least 20 randomly selected fields of outer cortex, and the mean number of PCT profiles per 20× high power field was calculated. The cortical thickness was determined by computer-assisted image analysis [Olympus BX-40 microscope (Olympus America, Melville, NY) interfaced via a CCD video camera (KP-161; Hitachi, Norcross, GA) to a Power Macintosh 7100/80AV (Apple Corp., Cupertino, CA) equipped with software for image analysis (Image NIH 1.59 for Macintosh; National Institutes of Health, Bethesda, MD)]. Kidney sections were further evaluated by periodic acid-Schiff (PAS) reaction and by immunohistochemistry for epithelial membrane antigen (EMA) (clone E29, Dako, Santa Barbara, CA) to aid in identification of PCT. The sections were examined by a pathologist blinded to the clinical status.

TUNEL labeling

Detection and quantitation of apoptotic cells was accomplished with terminal deoxynucleotidyl transferase-mediated dUTP-fluorescein isothiocyanate nick-end labeling (TUNEL), using the In Situ Cell Death Detection kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s instructions. Briefly, sections of kidney were dewaxed and rehydrated according to standard protocols, incubated with proteinase K (Sigma, St. Louis, MO; 20 µg/ml in 10 mmol/L Tris/HCl, pH 7.5 for 15 min at 37°C), rinsed and incubated with the TUNEL reaction mixture for 60 min at 37°C. Controls consisted of omission of the terminal transferase. The samples were washed in buffer and coverslipped using aqueous mounting medium containing DAPI. The TUNEL-positivity of PCT and medullary tubules was determined in