Adenosine Receptor Expression in the Development of Renal Fibrosis Following Ischemic Injury

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ABSTRACT

Long-term renal allograft survival has not improved despite improvements in short term outcomes. Graft loss is characterized histologically by the development of interstitial fibrosis and tubular atrophy (IFTA). Mechanisms underlying the development of IFTA are multifactorial and include ischemia-reperfusion injury (IRI). Therapeutic options to reduce IFTA include management of immunologic causes, such as rejection, but despite these efforts IFTA can still occur and leads to the inexorable destruction of the transplanted kidney. The adenosine A_{2B} receptor ($A_{2B}R$) has recently been implicated in the development of renal fibrosis. We performed an observational study to examine the mRNA expression of the adenosine receptors after renal ischemia up to the development of renal fibrosis in a mouse model of unilateral IRI. $A_{2B}R$ was the only adenosine receptor that showed elevated expression following ischemia until the development of renal fibrosis 4 weeks after injury. At 2 weeks after ischemia, increased expression of the fibrotic markers transforming growth factor β and Collagen-1α was observed. Expression of hypoxia inducible factor 1α and endothelin-1, which lie downstream of $A_{2B}R$ activation and have been recognized to promote renal fibrosis, were also significantly up-regulated at 2 weeks after ischemia. Expression of fibrotic markers returned to baseline by 4 weeks after ischemia, indicating resolution of injury with the concurrent development of renal fibrosis and reduced renal function. Our data suggest that $A_{2B}R$ may be a therapeutic target in reducing the development of renal fibrosis after ischemia.

RENAL transplantation is the preferred treatment for patients with end-stage kidney disease, but there is a large disparity between the availability of donor organs and the number of patients awaiting transplantation. In an attempt to close the gap and increase transplantation rates, the pool of donor organs has been expanded to include extended-criteria donors. Extended-criteria donors include older donors with comorbidities and donation after cardiac death. These donor kidneys often have increased warm and cold ischemia times, which together with donor factors increases the propensity of delayed graft function (DGF). Long-term graft survival is decreased in organs with DGF [1], and allograft loss is characterized histologically by interstitial fibrosis and tubular atrophy (IFTA), collectively known as renal fibrosis. IFTA or renal fibrosis is the end result of multiple allograft injury, including immunologic factors, chronic infection, drug toxicity, and early injury from ischemia-reperfusion injury (IRI).

There is evidence to suggest that adenosine signaling via the adenosine receptor $A_{2B}R$ mediates the development of renal fibrosis in mouse models of angiotensin-induced hypertension [2,3] and unilateral ureteric obstruction [3]. Lee et al [4] recently reported that the expression of all 4 adenosine receptors is up-regulated in the unilateral ureteric obstructive (UUO) rat model of renal fibrosis. Although the UUO model recapitulates the features of renal fibrosis, clinically obstructive nephropathy rarely contributes to allograft loss. Because IRI is obligatory in renal transplantation, we examined adenosine receptor expression after renal ischemic injury up to the development of renal fibrosis.

Extracellular adenosine is increased in inflammation and hypoxia [5], and signals through 4 G protein–coupled adenosine receptors, A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R [6].

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Adenosine signaling through A_1R, $A_{2A}R$, and $A_{2B}R$ is protective in acute ischemic injury to the kidneys [7], but $A_{2B}R$ appears to have opposing effects of promoting renal fibrosis [2,3]. In the model of angiotensin-mediated hypertension, the mechanisms of $A_{2B}R$-mediated renal fibrosis involved increased generation of endothelin-1 (ET-1), which is a potent vasoconstrictor, and increased expression of hypoxia-inducible factor 1α (HIF-1α). Inhibition or absence of $A_{2B}R$ reduced both ET-1 and HIF-1α expression, thereby reducing renal fibrosis [2]. It has been reported that hypoxia following ischemic injury is a potent stimulus for the generation of adenosine and up-regulation of $A_{2B}R$ and HIF-1α [5,8,9]. We therefore hypothesized that renal fibrosis following renal ischemia is mediated by $A_{2B}R$ and downstream increases in HIF-1α and ET-1 (Fig 1). To test this hypothesis, we performed an observational study to examine the mRNA expression of the adenosine receptors, HIF-1α, ET-1, transforming growth factor β (TGF-β), and a fibrotic marker, collagen-1α (Col-1α) in the kidney from 24 hours after ischemia to week 4 after injury. This observational study is the first step in delineating a role for $A_{2B}R$ in the development of renal fibrosis after ischemic injury.

MATERIALS AND METHODS

Animal Model of Unilateral Ischemic Injury

C57BL/6 mice were maintained in the Bioresources Centre of St Vincent's Hospital Melbourne (SVHM), and all procedures were approved by the SVHM Animal Ethics Committee. Male mice aged 10–14 weeks were anesthetized with the use of ketamine and xylazine (16 mg/kg and 8 mg/kg, respectively). The mice were then placed on a heat pad to maintain a core body temperature of 37°C during surgery. A midline laparotomy was performed, followed by right-sided nephrectomy. A microvascular clamp (Roboz, Rockville, Maryland) was applied to the left renal pedicle for 23.5 minutes while the mice were placed in a temperature-controlled chamber at 37°C. The clamp was then removed and the abdomen closed in 2 layers with silk-20 sutures. For hydration, each mouse received 200 μL warmed normal saline solution by intraperitoneal injection after the procedure. Sham-operated mice underwent right nephrectomy without ischemia. Cohorts of mice ($n = 3$–5) were killed at baseline, 24 hours, week 2, and week 4 after ischemia for tissue and serum analysis as described below. The ischemic time of 23.5 minutes was optimal for the development of renal fibrosis (data not shown). Ischemia for shorter periods did not result in renal fibrosis, and ischemia for longer periods resulted in high mortality. This is in keeping with a report that incomplete recovery from significant injury leads to the development of renal fibrosis [10].

Table 1. Gene Expression Assays Used in qRT-PCR

Gene	Taqman Probe
GAPDH	Mm99999915_g1
A_1R	Mm01308023_m1
$A_{2A}R$	Mm00802075_m1
$A_{2B}R$	Mm00839292_m1
A_3R	Mm01296602_m1
TGFβ	Mm01178820_m1
Collagen I	Mm00483888_m1
HIF-1α	Mm00468869_m1
Endothelin-1	Mm00438656_m1
CD39	Mm00515447_m1
CD73	Mm00501910_m1

Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; TGF, transforming growth factor; HIF, hypoxia-inducible factor.

Assessment of Renal Function

Whole blood was collected from the inferior vena cava, and serum creatinine levels were measured with the use of a modified Jaffe rate method with an Olympus AU2700 analyzer (Integrated Science, Chatswood, New South Wales, Australia) by the SVHM Department of Pathology.

Histologic Analysis

The left ischemic kidney was harvested and processed in 10% formalin and fixed in paraffin. Kidney tissue sections (3 μm) were stained with hematoxylin and eosin (H&E) to score the degree of tubular necrosis at baseline and at 24 hours. Tubular injury was scored in a blinded fashion based on the percentage of tubular necrosis in the cortex. Scores given were as follows: score 1: <10% of cortex with tubular necrosis; score 2: 10% to <25%; score 3: 25%–75%; score 4: >75%. This scoring system has been validated and published [11]. To detect renal fibrosis, kidney sections (3 μm) were stained with Masson trichrome. Slides were then scanned with the use of an Aperio Scanscope (Leica Biosystems, North Ryde, New South Wales, Australia) in a blinded fashion to generate a digitized image of the whole section. With the use of the positive pixel count algorithm, the area of fibrosis is measured as a fraction of the normal tissue. A semiquantitative morphometric score for the total area of fibrosis is then obtained. This method has been validated and published [12].

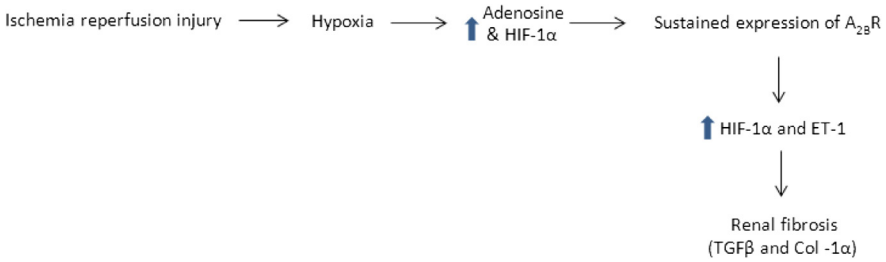

Fig 1. Proposed mechanism of adenosine receptor $A_{2B}R$-mediated renal fibrosis after renal ischemic injury. HIF, hypoxia-inducible factor; ET, endothelin; TGF, transforming growth factor; Col, collagen.