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Arterially Delivered Mesenchymal Stem Cells Prevent Obstruction-Induced Renal Fibrosis

Hiroshi Asanuma, M.D,* Brian A. Vanderbrink, M.D,* Matthew T. Campbell, M.D,* Karen L. Hile,* Hongji Zhang, M.D,* Daniel R. Meldrum, M.D,† and Kirstan K. Meldrum, M.D*,[1]

*Department of Urology; and †Department of Surgery, Indiana University School of Medicine, Indianapolis, Indianapolis, Indiana

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Background. **Mesenchymal stem cells (MSCs) hold promise for the treatment of renal disease. While MSCs have been shown to accelerate recovery and prevent acute renal failure in multiple disease models, the effect of MSC therapy on chronic obstruction-induced renal fibrosis has not previously been evaluated.**

Materials and Methods. **Male Sprague-Dawley rats underwent renal artery injection of vehicle or fluorescent-labeled human bone marrow-derived MSCs immediately prior to sham operation or induction of left ureteral obstruction (UUO). One or 4 wk later, the kidneys were harvested and the renal cortex analyzed for evidence of stem cell infiltration, epithelial-mesenchymal transition (EMT) as evidenced by E-cadherin/α-smooth muscle actin (α-SMA) expression and fibroblast specific protein (FSP+) staining, renal fibrosis (collagen content, Masson's trichrome staining), and cytokine and growth factor activity (ELISA and real time RT-PCR).**

Results. **Fluorescent-labeled MSCs were detected in the interstitium of the kidney up to 4 wk post-obstruction. Arterially delivered MSCs significantly reduced obstruction-induced α-SMA expression, FSP+ cell accumulation, total collagen content, and tubulointerstitial fibrosis, while simultaneously preserving E-cadherin expression, suggesting that MSCs prevent obstruction-induced EMT and renal fibrosis. Exogenous MSCs reduced obstruction-induced tumor necrosis factor-α (TNF-α) levels, but did not alter transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), interleukin-10 (IL-10), fibroblast growth factor (FGF), or hepatocyte growth factor (HGF) expression.**

Conclusions. **Human bone marrow-derived MSCs remain viable several weeks after delivery into the kidney and provide protection against obstruction-induced EMT and chronic renal fibrosis. While the mechanism of MSCs-induced renal protection during obstruction remains unclear, our results demonstrate that alterations in TNF-α production may be involved.**

Key Words: **mesenchymal stem cell; obstruction; epithelial-mesenchymal transition; fibrosis, kidney.**

INTRODUCTION

Obstructive nephropathy is a major cause of renal failure and end-stage renal disease in adults and children characterized by the development of tubulointerstitial fibrosis. Interstitial fibrosis is a complex pathophysiologic process involving inflammatory cell infiltration, fibroblast proliferation, and an imbalance in extracellular matrix (ECM) synthesis and degradation [1]. Activated fibroblasts are the principal effector cells responsible for extracellular matrix deposition and the development of tubulointerstitial fibrosis, and growing evidence suggests that growth factors, such as TGF-β1, can induce renal tubular epithelial cells to undergo phenotypic transformation into matrix producing myofibroblasts (EMT) under pathologic conditions [2, 3]. Renal cortical TGF-β1 levels increase significantly in response to obstruction [4], and evidence indicates that TGF-β1 is a major regulator of fibrosis *via* stimulation of EMT, fibroblast proliferation [5, 6] and extracellular matrix synthesis [1, 4, 6]. TNF-α also has a role in fibrotic renal injury, stimulating ECM accumulation and the up-regulation of a number

[1] To whom correspondence and reprint requests should be addressed at Department of Urology, Indiana University School of Medicine, Riley Hospital for Children, 702 Barnhill Drive, Suite 4230, Indianapolis, IN 46202. E-mail: kmeldrum@iupui.edu.

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of cytokines and transcription factors involved in tubulointerstitial fibrosis, including TGF-β1 [7].

MSCs are undifferentiated non-embryonic stem cells of mesodermal origin that have the capacity to differentiate into cells of connective tissue lineage [8]. While MSCs have the capacity to differentiate, recent studies suggest that a more significant component of their protective and reparative function resides in their paracrine activity. MSCs can secrete a number of growth factors and cytokines that are important for angiogenesis and cytoprotection, including VEGF, TGF-β1, FGF, and HGF [9, 10]. VEGF is involved in matrix remodeling, monocyte chemotaxis, and adhesion molecule expression, and stimulates the proliferation of peritubular capillaries that are essential for tubular regeneration [11]. FGF-2 is intimately involved in angiogenesis and may be a more potent angiogenic factor than VEGF [12]. HGF improves cell growth, reduces cell apoptosis, and exerts a beneficial effect on neovascularization and tissue remodeling [13]. While a number of studies in pathologic models of kidney disease have shown that exogenous MSCs can home to injured kidneys and facilitate repair, the effect of MSCs therapy on chronic obstruction-induced renal fibrosis has not previously been evaluated. This study therefore evaluated (1) the localization of arterially injected MSCs during renal obstruction, (2) the effect of exogenous MSCs on obstruction-induced EMT and renal fibrosis, and (3) the effect of exogenous MSCs on obstruction-induced renal cytokine and growth factor activity in rat model of UUO.

MATERIALS AND METHODS

MSC Labeling

Human MSCs were purchased from Cambrex Bio Science Walkersville, Inc. (Walkersville, MD), and cultured in MSC Basal Medium (Lonza, Walkersville, MD) containing 10% fetal bovine serum for 14 d prior to injection. According to the manufacturer, cells are positive for CD105, CD166, CD29, and CD44, and negative for CD14, CD34, and CD45. MSCs were labeled with the PKH26 red fluorescence cell linker kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's protocol, just prior to injection.

Animals, Experimental Groups, and Operative Techniques

The animal protocol was reviewed and accepted by the animal care and research committee at Indiana University School of Medicine. Adult male Sprague-Dawley rats weighing 250 to 300 g were acclimated and maintained on a standard pellet diet for 1 wk before experiment initiation. Rats were anesthetized using isoflurane inhalation. Following the induction of anesthesia, rats underwent renal arterial injection of either vehicle or MSCs (1×10^6 per rat). The left ureter was subsequently ligated to induce obstruction. Sham treated animals underwent an identical surgical procedure without ureteral ligation. Animals (six per group) underwent renal arterial injection of fluorescently tagged MSCs *versus* vehicle immediately prior to UUO and were subjected to 1 or 4 wk of obstruction *versus* sham operation. The kidneys were subsequently removed, snap frozen in liquid nitrogen, and analyzed for MSC localization and markers of renal fibrosis.

The animals were divided into three experimental groups (six animals per group): 1-wk sham operation (Sham), 1-wk UUO plus vehicle (OB), and 1-wk UUO plus MSCs (OB + SC).

MSC Localization

In order to examine MSC localization following arterial delivery, kidney samples were prepared from each time point (1 wk, 4 wk) with a cryostat and fixed in 4% methanol-free formaldehyde for 15 min. MSCs were labeled with PKH26 as described above. The slides were then washed three times in PBS for 5 min and the nuclei counterstained with bisbenzimide for 30 s. The slides were then washed and mounted with ProLong Antifade (Molecular Probes, Eugene, OR), and the sections photographed. Nonspecific fluorescence was determined from vehicle treated sections and digitally subtracted from MSC treated sections and samples were then photographed using a fluorescent microscope (400×; Leica DM IRB, Wetzlar, Germany).

Tissue Homogenization

A portion of the renal cortex from each kidney was homogenized following dilution in five volumes of homogenate buffer composed of 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM egtazic acid, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride, using a VirTishear tissue homogenizer (VirTis, Gardiner, NY). Renal homogenates were centrifuged at 3000 *g* for 15 min at 4°C. The supernatants were subsequently stored at –80°C until further testing could be performed.

Western Blot Analysis

Protein extracts (30 μg per lane) from homogenized samples were electrophoresed onto a tris-glycine gel (Invitrogen, Carlsbad, CA) and transferred to polyvinylidene fluoride membrane. Immunoblotting was performed by incubating each membrane in 5% dry milk for 45 min at room temperature, followed by incubation with an anti-rat E-cadherin (1:500; BD Biosciences, San Jose, CA) or an anti-rat α-SMA (1:500; Sigma-Aldrich, St. Louis, MO) antibody overnight at 4°C. After being washed three times in tris-PBS each membrane was incubated for 1 h with a peroxidase-conjugated secondary antibody (1:2,000 for E-cadherin, 1:2,000 for α-SMA) at room temperature. The membranes were then developed using enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ). Equivalent protein loading for each lane was confirmed by stripping and reblotting each membrane for GAPDH (1:20,000 for 30 min at room temperature, secondary 1:20,000 for 30 min at room temperature; Biodesign International, Saco, ME). The analysis was repeated in triplicate to ensure the reproducibility of results. The density of each band was determined using NIH image analysis software and expressed as a percent of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) density.

Fibroblast (FSP-1) Accumulation

Renal tissue sections were analyzed for the presence of transformed fibroblasts (EMT) utilizing an S100-A4 antibody (The S100-A4 antigen is also known as FSP-1) [2, 14]. Transverse 4 μm renal tissue sections were deparaffinized and dehydrated with xylene and alcohol. Antigen was retrieved by incubating the cells with proteinase K for 20 min in an oven. The tissues were then blocked with 1% bovine serum albumin and incubated with a polyclonal rabbit antibody directed against S100-A4 (FSP-1, 1:200; DAKO Cytomation, Carpinteria, CA) for 1 h at 37°C. Sections were washed and incubated with a secondary antibody (goat anti-rabbit; 1:50) for 30 min. Peroxidase-stained sections were then developed with