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# Protective effects of mesenchymal stromal cells on adriamycin-induced minimal change nephrotic syndrome in rats and possible mechanisms

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**Abstract**

*Background aims.* Minimal change nephrotic syndrome is the most frequent cause of nephrotic syndrome in childhood. Current treatment regimes, which include glucocorticoid hormones and immunosuppressive therapy, are effective and have fast response. However, because of the side effects, long treatment course, poor patient compliance and relapse, novel approaches for the disease are highly desired. *Methods.* The adriamycin-induced nephrotic rat model was established. Rats were allocated to a model group, a prednisone group or mesenchymal stromal cell (MSC) group. Clinical parameters in each treatment group were determined at 2 weeks, 4 weeks and 8 weeks. The messenger RNA (mRNA) levels of synaptopodin, p21 and monocyte chemoattractant protein-1 were determined through the use of quantitative real-time–polymerase chain reaction. Protein levels were determined by means of Western blot or enzyme-linked immunosorbent assay. Podocytes were isolated and apoptotic rate after adriamycin with or without MSC treatment was analyzed by means of flow cytometry. *Results.* MSC intervention improved renal function as assessed by urinary protein, blood creatinine and triglyceride levels. MSC intervention reduced adriamycin-induced renal tissue damage visualized by immunohistochemistry and light and electron microscopic analysis and reduced adriamycin-induced podocyte apoptosis. After MSC intervention, mRNA and protein levels of synaptopodin and p21 in renal cortex were significantly increased. MSCs also restored synaptopodin mRNA and protein expression in isolated podocytes. In addition, monocyte chemoattractant protein-1 mRNA in renal cortex and protein level in serum of the MSC treatment group were significantly decreased compared with that in the adriamycin-induced nephropathy model group. *Conclusions.* Our data indicate that MSCs could protect rats from adriamycin-induced minimal change nephrotic syndrome, and the protective effects of MSCs are mediated through multiple actions.

**Key Words:** *mesenchymal stromal cells, nephrotic syndrome, p21, synaptopodin*

## Introduction

Minimal change nephrotic syndrome (MCNS) is characterized by podocyte abnormalities and proteinuria (1,2) and is the most frequent cause of nephrotic syndrome in childhood. Major clinical manifestations include foamy appearance of the urine, massive proteinuria, poor appetite, swelling (around the eyes, feet and ankles), bloating in the abdomen and weight gain. Although the pathology of this disease currently remains unclear, this disease is proposed to be related to the glomerular permeability, the podocyte immunity (3), T-cell dysfunction and cell-mediated immune function disorders (4–6).

Podocytes are highly specialized and terminally differentiated epithelial cells that overlay the outer aspect of the glomerular basement membrane (7). Podocytes play a central role in renal physiology, including the prevention of proteinuria. Foot processes are several primary processes that arise from the cell body. The glomerular slit diaphragm (SD) represents the junction structure that links the interdigitating foot processes from neighboring podocytes. Podocytes and the SD complex serve as the final barrier against protein leakage from the kidney (8,9). Podocyte abnormality and injury and SD damage are generally believed to be associated with massive

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proteinuria (10–13), which often leads to renal fibrosis and sclerosis, and accelerates the progression to end-stage renal disease. Slit diaphragm proteins or podocyte cytoskeleton proteins, such as synptopodin, nephrin and podocin, are crucial for maintaining the integrity of the filtration barrier (1,14).

At present, glucocorticoid hormones (15) and immunosuppressive therapies (16) are generally administered to patients with MCNS. These approaches are effective and provide fast response; however, side effects, long treatment course, poor patient compliance and relapse are the downsides of the treatments (17). Novel non-hormone treatments for the disease are highly desired.

Stem cell–based approaches have enormous potential for the development of future therapies (18). Accumulated data in recent years have demonstrated the therapeutic capacity of different stem cell populations. Mesenchymal stromal cells (MSCs) are stem cells that can differentiate into mesenchymal tissues. Their stem cell properties include self-renewal, multi-lineage differentiation and homing ability. MSCs are mainly derived from bone marrow, periosteum, cancellous bone, adipose tissue, synovium, skeletal muscle and umbilical cord. Previous studies have shown the protective effects of MSCs during renal injury. Administration of MSCs has provided protection in several models of acute kidney injury (19–21). Two studies demonstrate a positive effect of MSC treatment on the loss of renal function (protein/creatinine ratio) in chronic kidney disease models (22,23). The functional improvement in various renal diseases after administration of MSCs was explained on the basis of the paradigm of engraftment and differentiation. Recently, English *et al.* (24) found that MSCs have immunomodulatory properties, including suppression of T-cell and B-cell proliferation, influence on dendritic cell maturation and function, suppression of B-cell terminal differentiation and immune modulation of natural killer cells and macrophages. These findings have important implications because immune cell dysfunction may be crucial for the development of MCNS or other chronic kidney disease (4–6). In addition, Kunter *et al.* (25) showed in a mesangial proliferative glomerulonephritis model that MSCs may slow the progress of acute renal failure through excretion of high concentrations of cytokines, such as vascular endothelial growth factor and transforming growth factor-$\beta$. Their paracrine action may reduce tubulointerstitial fibrosis, decrease glomerular adhesion and protect the glomerular function. Acumulated lines of evidence also demonstrated that MSCs exert various other functions including immune regulation (26) and tissue remodeling and repairing in nephritic injury models (27–29). The diverse functions of MSCs enable them to play crucial roles in improving the proteinuria condition in various renal diseases.

Although no animal model completely mimics MCNS in humans, the adriamycin-induced nephropathy (AN) model shares multiple characteristics with MCNS within 4 weeks of onset (30) and progresses to focal segmental glomerular sclerosis by 9 to 12 weeks. Similar to MCNS, AN shows high proteinuria; hyperlipidemia by 2 weeks; edema, diffuse fusion and didappers by 4 weeks; and focal segmental glomerular sclerosis by 8 weeks, which continues to deteriorate. AN also involves podocyte injury followed by tubulointerstitial inflammation and fibrosis. In the present study, we used the adriamycin-induced nephrotic injury rat model (MCNS/focal segmental glomerular sclerosis) to investigate the effects of MSCs on disease progression and possible mechanisms. These findings provide new insights into the mechanisms of MSC-based therapy and thus can be of assistance in the development of new therapeutic regimens for minimal change nephrotic syndrome.

## Methods

The research protocol conducted for the animal experiments in this study was approved by the Institutional Animal Committee of Fuzhou General Hospital, Xiamen University (Fuzhou, China).

### *Materials*

Healthy and clean grade male Sprague-Dawley rats (3–4 months of age; weight, 200 ± 20 g) were purchased from SLAC Laboratory Animal Shanghai, China. Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM)-LG and trypsin were purchased from Hyclone (Thermos Fischer Scientific, Beijing, China).

### *Isolation and characterization of bone marrow MSCs*

Bone marrow–derived MSCs (BMSCs) were isolated, cultured and characterized, with routine laboratory operation procedures followed (31). Briefly, the femurs and tibiae were removed from immature male Sprague-Dawley rats. The bone marrow plugs were flushed with phosphate-buffered saline (PBS), which was layered over a Percoll solution (density, 1.083) and separated by centrifugation at 1000*g* for 20 min at room temperature. Mononuclear cells at the interface were recovered and washed twice with PBS. The cells were cultured with complete medium, consisting of DMEM/F12 (1:1) medium