Human Umbilical Cord-Derived Mesenchymal Stem Cells
Conditioned Medium Attenuate Interstitial Fibrosis and Stimulate the Repair of Tubular Epithelial Cells in an Irreversible Model of Unilateral Ureteral Obstruction

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Conditioned medium in renal interstitial fibrosis

ABSTRACT:

Aim: The growing number of patients suffering from chronic renal disease (CKD) is a challenge for the development of innovative therapies. Researchers have studied the therapeutic effects of cell therapy in acute kidney injury (AKI). However, the therapeutical effect of conditional medium (CM) in the CKD models have been rarely reported. Here, we examined the effects of umbilical cord derived-mesenchymal stem cells (hUC-MSCs) CM on renal fibrosis in a rat model of unilateral ureteral obstruction (UOO).

Methods: We randomly divided the animals into three groups: sham-operated, UOO, UOO + CM. CM was administered via the left renal artery after total ligation of the left ureter. Rats were killed after 14 days of obstruction. Histological changes and oxidative stress parameters were assessed. Western blotting and immunohistochemistry analysis were used to measure epithelial-mesenchymal transition (EMT) markers, including epithelial cadherin (E-cadherin), α-smooth muscle actin (α-SMA), tumor necrosis factor-α (TNF-α), Collagen-I, and transforming growth factor β1 (TGF-β1). Proliferation and apoptosis of renal tubular epithelial cells (RTEs) were also measured.

Results: HucMSC-CM significantly reduced the levels of malondialdehyde (MDA) and reactive oxygen species (ROS), and increased the activity of glutathione (GSH) induced by
UUO. Moreover, CM significantly reduced the expression of TGF-β1, α-SMA, TNF-α and Collagen-I in UUO kidney, promoted the proliferation of RTEs and inhibited its apoptosis. In addition, the increased expression of E-cadherin also reflects the effective improvement of renal interstitial fibrosis.

**Conclusion:** This study shows that CM protects UUO-induced kidney damage and therefore could be a potential tool to prevent CKD progression.

**Key words:** Mesenchymal stem cells (MSCs); Fibrosis; Unilateral ureteral obstruction (UUO); Repair

**INTRODUCTION**

As a word public health concern, chronic kidney disease (CKD) has attracted wide attention from researchers [1]. In fact, each type of CKD is associated with increased activity of myofibroblasts and excessive accumulation of extracellular matrix (ECM), which inevitably result in interstitial fibrosis. End-stage renal failure caused by renal interstitial fibrosis is a progressive process that requires dialysis or kidney transplantation [2]. Since most of the kidney diseases (including acquired, congenital, interstitial, glomerular, etc.) at the final stage is associated with renal interstitial fibrosis, recognizing the underlying pathology factors and contributors of this process to prevent or reverse these changes is of great importance.

Unilateral ureteral obstruction (UUO) is an established model of renal tubulointerstitial fibrosis. Rabbit UUO was demonstrated to cause renal interstitial fibroblasts proliferation and conversion into myofibroblasts, while fibronectin, interstitial collagens I, III, IV, and heparin sulfate proteoglycan are increased [3]. Ureteral obstruction after a few days can cause
hydronephrosis, interstitial damage, inflammatory cell infiltration, renal tubular epithelial cell apoptosis and necrosis [4]. Therefore, the UUO model is suitable for studying the pathological and molecular mechanisms of renal interstitial injury because it can mimic the deterioration of renal function in human chronic kidney disease [5]. At present, the UUO animal model has not only been widely used to elucidate the pathogenesis of obstructive nephropathy, but also widely used to study the mechanism of progressive renal fibrosis in CKD. [6].

Because of the great potential for clinical applications, stem cell-based therapies have attracted a lot of attention from the researchers. Previous studies have shown that MSCs may play a protective role in different types of kidney damage by trans-differentiation or secreting paracrine factors [7, 8]. Nonetheless, the incorporation and trans-differentiation of injected MSCs were rare events, which indicates that MSCs were most likely to play a supporting role by secreting growth factors and cytokines. This paracrine function laid the foundation for the use of MSC-secreted factors in cell-free treatment strategies. More importantly, if the factors secreted by the MSC can slow the progression of CKD, this can overcome the drawbacks of allogeneic MSC transplantation, such as inconvenience, immunological rejection, and MSC tumorigenesis [9]. Previous studies have shown that MSC-CM promotes proliferation and migration of alveolar epithelial cells under septic conditions [10]. However, few studies have been specially designed to study the effects of MSC secretion factors in kidney diseases. Human umbilical cord mesenchymal stem cells (hUC-MSCs) are a prospective source for cell therapy which shows capable of self-renewing, multilineage differentiation, homing abilities, and paracrine properties [11]. In this study, hUC-MSCs condition medium (CM) was infused
into Sprague Dawley (SD) rats with UUO, the effect of transplanted hUC-MSCs condition medium on the apoptosis and proliferation of renal tubular epithelial cells in CKD model and the effect on interstitial fibrosis were evaluated.

METHODS

Ethics statement

This study was approved by the Research Ethics Committee of Chongqing Medical University. Twenty-four male SD rats weighing 180 - 200 g were provided by Chongqing Medical University Experimental Animal Center [SPF, License No.: SYXK (Chongqing) 2007-0001] and were housed in the Chongqing Medical University Children’s Hospital Experimental Animal Center [SPF, License No.: SYXK (Chongqing) 2007-0016]. The rats were housed in polycarbonate cages under a guarded environment with a 12 hour light/dark illumination schedule, free of water or food.

Preparation of hucMSCs conditional medium

The normal hucMSCs was purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Eighty percent confluent hucMSCs were washed twice with PBS buffer and then incubated for 24h with low glucose serum-free DMEM medium (HyClone, USA). The supernatants in each dish was collected and centrifuged at 1,200 rpm for 5 min, filtered with a 0.22-μm filter (Merck KGaA, Darmstadt, Germany) to remove cellular debris. For each SD rat, we used 500μl of conditioned medium generated by approximately $5 \times 10^6$ cells.
UUO and Injection of hUCMSC-CM

SD rats were randomly divided into three groups (n=8 for each group), including Sham operation group (Sham group), UUO group, UUO combined with CM group (UUO + CM group). The animals in the sham operation group received an identical surgical process in addition to ureteral ligation. The CM group received hUC-MSC conditional medium (500μl) via left renal artery after the surgery. Briefly, 10% Chloral hydrate (3 ml/kg) was used to anesthetize the male SD rats. A flank incision was used to expose the left kidney and the ureter. Next, the left ureter was ligated with two 4-0 silk sutures at the ureterovesical junction. In order to avoid the urinary tract retrograde infection, the left ureter was cut between the knots. Animals in all groups received antibiotics (0.1% amoxicillin) for 14 days in their drinking water. At 14 days postoperatively, the animals were euthanized and the left kidneys were extracted. For each kidney, one portion was fixed with 4% paraformaldehyde for histological examinations and immunohistochemical staining, and the remainder was stored in liquid nitrogen for the following experiment.

Histological examinations

The specimens in each group were fixed in paraformaldehyde and then paraffin-embedded, sectioned into 4 μm thick. Hematoxylin-eosin and periodic acid-Schiff (PAS) were then used to stain the sections. The renal tubular injury was scored according to the scale of 0–3: 0 = normal histomorphology, 1 = mild dilation, 2 = epithelial cells become flattened, brush border lost, and 3 = basement membrane exposed, tubular epithelial cell apoptosis and necrosis. The scoring of tubular injury was conducted by two pathologists in a blinded manner. A total of 200 renal tubules were assessed for each slice, and the mean score for all renal tubules was
the total score. [12]. Interstitial fibrosis was detected by Masson’s trichrome staining, and collagen fibers were stained blue. Under higher-power magnification (×40), 10 discontinuous visual fields of the renal outer cortex were randomly selected in each slice by using microscope (Nikon, Japan). The analysis did not include large blood vessels and glomeruli. The percentage of fibrotic area in total area was calculated by using Image 6 Pro Plus System (Media Cybernetics, USA).

Assay of GSH, MDA, and ROS level

The effect of CM on glutathione (GSH) level was measured by a commercial kit (Cayman Chemical Company, USA), according to the manufacturer’s protocol, kidney tissue homogenate was used for GSH determination. The lipid peroxidation of renal tissue was studied by measuring the level of malondialdehyde (MDA). The formation of TBA adduct was determined by colorimetric method according to the method provided by TBARS Assay Kit (Cayman chemical Company, USA). The production of intracellular reactive oxygen species (ROS) was quantified via the DCFH-DA probe (Sigma-Aldrich, USA). The DCF standard curve was used to quantify the ROS formation, the data was expressed as pmol DCF formed/min/mg protein [13].

Immunohistochemical analysis

Briefly, after dewaxing, antigen repair and blocking of endogenous antigens, the sections were incubated overnight at 4°C with monoclonal mouse anti-E-cadherin antibodies (1:200) (Santa Cruz, USA), polyclonal rabbit anti-TGF-β1 antibodies (1:200) (Abcam, USA), monoclonal mouse anti-α-SMA antibodies (1:200) (Abcam, USA), polyclonal rabbit anti-TNF-α antibodies (1:200) (Boster Biotechnology, China), and polyclonal rabbit
anti-Collagen-I antibodies (1:200) (Boster Biotechnology, China) respectively. After washed three times with PBS, the sections were incubated for 1 hour at 37°C with the corresponding secondary antibody, tested with a DAB kits (Zhongshan, China), and finally stained with hematoxylin.

**Western blot analysis**

Renal samples were lysed in RIPA Lysis Buffer (Beyotime, China) and centrifuged at 4°C at 12,000 rpm for 20 min. The protein concentration was measured using the BCA assay kit (Beyotime, China). SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) loading buffer was mixed with protein samples and boiled last for 10 min. The protein were electrophoresed through SDS-polyacrylamide gels. After that, proteins were transferred to PVDF (Polyvinylidene fluoride) membranes (Millipore, USA). In order to block nonspecific protein background, membranes were blocked in 5% skim milk for 1 hour with shaker. The protein bands were then incubated with monoclonal mouse anti-E-cadherin antibodies (1:1000), polyclonal rabbit anti-TGF-β1 antibodies (1:500), monoclonal mouse anti-α-SMA antibodies (1:200), polyclone rabbit anti-Collagen-I antibodies (1:200), polyclone rabbit anti-TNF-α antibodies (1:200) overnight at 4°C. The membrane was then washed three times with Tris-buffered saline/Tween (TBST) and incubated in goat anti-rabbit or mouse antibodies (Zhongshan, China) for 2 hours at 37°C. Immobilon Western Chemiluminescent HRP Substrate (Millipore, USA) were used to detect positive immune reactions. The immunoreactive bands were quantified by densitometric analysis (GeneGnome, USA) and standardized with β-actin.

**Proliferation and Apoptosis measurements**
PCNA staining Kit (Invitrogen, USA) and TUNEL Apoptosis Detection Kits (Beyotime, China) were used to detect renal tubular epithelial proliferating and the number of apoptotic cells. All staining procedures were carried out in accordance with the instructions provided by the manufacture. In each experimental group, the proliferating/apoptotic nuclei in all the nuclei in the slice was calculated using a consecutive, non-overlapping field of view in the stained specimens.

**Statistical analysis**

All data are asserted as mean ± standard deviation (mean ± SD). The differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparision test. Differences were considered to be statistically significant if the p-values were less than 0.05 ($P < 0.05$).

**RESULTS**

**Hematoxylin-eosin, Periodic acid-Schiff and Masson’s trichrome staining of Renal Tissues**

As shown in Figure 1, there was no obvious histological abnormality in the sham group (Figure 1A). At 14 days after UUO, renal tubular dilation or atrophy, inflammatory cell infiltration, vascular collapse, extracellular matrix deposition, and significant interstitial fibrosis were observed (Figure 1B). However, the degree of renal injury in CM group was less than that in UUO group (Figure 1C). To assess the number and extent of damaged renal tubules, sections were stained with PAS. In the sham group, the proximal tubules and the distal tubules remain intact (Figure 1D). At 14 days after UUO, the structure of the proximal tubule and the distal tubule was disordered, accompanied by protein casts and brush border.
destruction (Figure 1E). However, the number and extent of damaged renal tubules in the CM group were less than those in the UUO group \((P < 0.05)\) (Figure 1F, J). Masson’s trichrome staining showed that few collagens were deposited around renal tubules in the sham group (Figure 1G). At 14 days postoperatively, a lot of collagen fibers were seen in the renal interstitial region (Figure 1H). However, the interstitial area of CM group was significantly lower than that of UUO group \((P < 0.05)\) (Figure 1I, K).

**Effect of CM on oxidative stress in kidney**

Compared with the sham group, the UUO group showed a significant increase in MDA and ROS levels, and CM transplantation significantly reduced the amount of MDA and ROS in the UUO kidney \((P < 0.05)\). The amount of GSH in the UUO group was significantly reduced. However, the amount of GSH in the renal tissue was significantly increased compared with the UUO group \((P < 0.05)\) (Fig. 2).

**Immunohistochemical staining and western blot analysis of E-cadherin**

Immunohistochemical staining showed that E-cadherin in sham group was mainly expressed on the cell membrane of renal tubular epithelial cells (Figure 3A). At 14 days postoperatively, the renal tubules in UUO were significantly destroyed and the expression of E-cadherin was decreased (Figure 3B). Although the expression levels of E-cadherin in CM group was also lower than that in sham group, it was still significantly higher than that in UUO group (Figure 3C). Western blot analysis of E-cadherin in each group was consistent with the findings of immunohistochemical staining \((P < 0.05)\) (Figure 3P, Q).

**Immunohistochemical staining and western blot analysis of TGF-β1**

Immunohistochemical staining showed slight TGF-β1 expression in the sham group (Figure
3D). However, at 14 days postoperatively, a significant increase in TGF-β1 staining was observed in the renal tubules, renal interstitial regions, and glomeruli (Figure 3E). The expression of TGF-β1 in CM group was significantly lower than that in UUO group (Figure 3F). Western blot analysis of TGF-β1 in each group was consistent with the findings of immunohistochemical staining ($P < 0.05$) (Figure 3P, R).

**Immunohistochemical staining and western blot analysis of α-SMA**

Immunohistochemical staining showed that α-SMA positive staining was seen only around the blood vessels in sham group at 14 days postoperatively (Figure 3G). However, in the 14 days after UUO, a large number of α-SMA positive staining was seen in the renal interstitium (Figure 3H), while the area of α-SMA positive staining in the CM group was significantly reduced (Figure 3I). Consistent with the immunohistochemical results. Western blot analysis of α-SMA in each group was consistent with the findings of immunohistochemical staining ($P < 0.05$) (Figure 3P, S).

**Immunohistochemical staining and western blot analysis of TNF-α**

To determine whether the CM can manage the inflammatory response of UUO, the proinflammatory cytokine TNF-α was analyzed by immunohistochemical staining and western blot. In the sham group, slight TNF-α staining was seen in the renal interstitium (Figure 3J). Furthermore, Immunohistochemical staining showed that the expression of TNF-α was significantly increased in the UUO group compared with the sham group (Figure 3K). However, CM transplantation can significantly reduce the expression of TNF-α in renal tubular epithelial cells after UUO surgery (Figure 3L). Western blot analysis of TNF-α in each group was consistent with the findings of immunohistochemical staining ($P < 0.05$)
Immunohistochemical staining and western blot analysis of Collagen-I

In the sham group, slight collagen I staining was seen in the renal interstitium (Figure 3M). Furthermore, the expression level of collagen I in the UUO group was significantly higher than that in the sham group, and concentrated in the interstitial region (Figure 3N). However, CM transplantation can significantly reduce the expression of collagen I after UUO surgery (Figure 3O). Western blot analysis of collagen I in each group was consistent with the findings of immunohistochemical staining ($P < 0.05$) (Figure 3P, U).

Proliferation and Apoptosis

At 14 days postoperatively, the proliferation and apoptosis of renal tubular epithelial cells were compared between groups. In the sham group, there was no significant increase in the proliferation of renal tubular epithelial cells (Figure 4A). On the contrary, the cell proliferation was significantly enhanced in the UUO group (Figure 4B), while the cell proliferation in the CM group was significantly higher than that in the UUO group (Figure 4C, G). In addition, in the sham group, no significant renal tubular epithelial cell apoptosis was observed (Figure 4D), and UUO group can be seen serious destruction of renal tubular associated with a lot of apoptotic cells (Figure 4E). However, the number of apoptotic cells in CM group was significantly lower than that in UUO group (Figure 4F, H).

DISCUSSION

The characteristic pathologic changes in UUO include tubular dilatation or atrophy, interstitial inflammatory cell infiltration, fibroblast proliferation, extracellular matrix deposition, and renal interstitial fibrosis [14]. Renal interstitial fibrosis is a common pathway
for chronic kidney disease progression to end-stage renal disease, and there is still a lack of effective treatment to prevent the development of renal interstitial fibrosis. End-stage renal disease can significantly increase the risk of cardiovascular disease, requiring special health care and even cause death. Therefore, it is imperative to find a new and feasible method for the treatment of CKD.

Over the past few decades, cell-based therapy has been used to repair kidney damage. Many studies have shown that MSC transplantation can repair damaged kidneys both in experimental models and clinical setting [7, 8, 15]. However, the reason that impede the use of these cells include susceptibility to embolism, tumor risk, and high immunity. Although hUC-MSCs have low immunogenicity and immunosuppressive functions can alleviate acute immune rejection [16]. However, the risk of maldifferentiation and tumorigenesis cannot be avoided. The condition medium of stem cells has succeeded in avoiding these risks and has become a potential source of clinical use. In this study, we evaluated the therapeutic effect of CM on UUO-induced chronic kidney disease.

Reactive oxygen species (ROS) plays an important role as a key signaling molecule in the progress of interstitial fibrosis and epithelial-mesenchymal transition (EMT). Recent studies have shown that ROS also plays a role in the UUO rat mode [17]. Our study also showed a significant increase in the amount of ROS in the kidneys of the UUO group compared to the sham group. MDA is the product of oxidative stress in vivo, which can reflect the level of oxidative stress. We found that the level of MDA in the kidney of UUO rats was significantly increased, while that of CM could reduce the production of MDA in the kidney. GSH is an important antioxidant and free radical scavenger, and the results found that CM can
significantly slow down the decline in GSH in UUO kidney. Thus, CM may play a role in protecting the kidneys of CKD by restoring the balance of redox reactions in the UUO kidney.

Previous studies have shown that ROS also has an effect on the inflammatory microenvironment [18]. TNF-α is an important inflammatory factor in the pathophysiology of various kidney diseases. TNF-α directly promotes UUO-induced apoptosis of renal tubular epithelial cells, collagen deposition and interstitial fibrosis. Previous study have shown that altered TNF-α production may be a mechanism of MSC-induced renoprotection during UUO [19]. In our study, the level of TNF-α was significantly increased of the UUO rat kidney. However, CM transplantation can reduced the production of TNF-α, suggesting that CM can reduce the inflammatory response caused by renal injury, thereby reducing renal tubular injury.

A hallmark of EMT is down-regulation of E-cadherin, which has been considered as an epithelial marker in most EMT-related researches [20]. In our study, although the expression of E-cadherin in UUO group and CM group was lower than that in sham group, the expression of E-cadherin in CM group increased compared with UUO, suggesting that CM could inhibit the morphological changes of UUO kidney and further improve renal tubular damage. TGF-β1 is an important cytokine for the induction of the EMT in renal fibrosis, which interfere renal fibrosis by causing the conversion of tubular epithelial cells (TECs) to myofibroblasts through EMT. In addition, TGF-β1 also mediates the progression of renal fibrosis by stimulating the production of extracellular matrix (ECM) and inhibiting its degradation [21]. Our results showed that the expression of TGF-β1 in UUO group was
significantly higher than that in sham group. However, CM transplantation significantly reduced the expression of TGF-β1 in UUO kidney.

After UUO, renal tubular epithelial cells lost epithelial cells markers (E-cadherin) that began to express marker of mesenchymal cells marker (α-SMA), which implicates that epithelial cells were transformed into myofibroblasts. However, this process was inhibited by CM transplantation. The balance of collagen synthesis and degradation in UUO kidney was broken [22], renal interstitial cells produced excessive collagen I, causing renal interstitial fibrosis. Our findings also confirmed a significant increase in collagen I in the kidneys of the UUO group at 14 days postoperatively. The expression of collagen I in CM group was significantly lower than that in UUO group, suggesting that CM could reduce collagen I deposition in UUO kidney and further improve renal interstitial fibrosis. Finally, in order to assess the effect of CM transplantation on renal tubular epithelial repair, we analyzed the proliferation and apoptosis of renal tubular epithelial cells in each group. The results showed that CM transplantation could accelerate the repair of renal tubular epithelial cells damaged by UUO and inhibit the apoptosis of renal tubular epithelial cells.

Previous studies have shown that MSC can play an important role in anti-inflammatory, anti-oxidative and anti-fibrosis after entering the UUO rat kidney. The detection of the distribution of labeled MSC in the kidneys revealed that the actual number of MSCs remaining in the kidneys was low, and the researchers hypothesized that paracrine secreted by MSC played an important role in repairing kidney damage [20]. In our study, we found that CM also has the ability to regulate the inflammatory response of the UUO kidney, reduce oxidative stress injury, reduce collagen deposition, slow down the EMT process, promote
renal tubular epithelial cell proliferation, and inhibit renal tubular epithelial cell apoptosis. Similar results were also observed in the repair of other renal injury models by using MSC-CM. For instance, the results of Koopen have shown that MSC-CM has the effect of slowing the progression of CKD in 5/6 nephrectomy rats, increasing glomerular filtration rate, lowering systolic blood pressure, and reducing proteinuria production [23]. Another study also showed that CM of stromal cells could reduce cisplatin-induced proximal tubule epithelial cell apoptosis and promote the proliferation of tubule epithelial cells [24]. However, the present study is the first time to measure the impact of arterially delivered hUC-MSC condition medium on obstruction-induced EMT as well as renal fibrosis.

Recent reports have shown that microvesicles (MV) or exosomes plays a central role in MSC-mediated tissue repair. In experimental ischemia/reperfusion injury. Lai et al. have confirmed that exosomes secreted by MSC were involved in myocardial protection of myocardial ischemia-reperfusion injury [25]. The results of Lee’s research suggested that vascular remodeling can be inhibited by exosomes derived from mouse MSC conditioned medium in a model of hypoxic pulmonary hypertension. In addition, the results also found that exosomes increase the right ventricular systolic pressure by suppressing the hypoxic pulmonary influx of macrophages and the induction of pro-inflammatory and pro-proliferative mediators [26]. In the kidney, MSC derived MV can effectively improve kidney damage caused by glycerol-induced acute kidney injury (AKI) and ischemia-reperfusion injury (IRI) [27, 28]. This theory could explain why, in our study, CM transplanted UUO rats had a reduced severity of kidney damage. Thus, we speculate that paracrine factors carried by exosomes and microvesicles generated by a large number of cells
in vitro may play an important role injury repair in vivo.

In summary, our study firstly shows that via renal artery administration of hUC-MSCs condition medium can reduced inflammation, oxidative stress, and collagen deposition, slow the progress of EMT, reduce renal interstitial fibrosis, promote tubular epithelial cells proliferation and inhibit its apoptosis in UUO rats. These results indicate a potential approach of treating renal fibrosis and CKD. However, the mechanisms by which MSC-CM regulates the responses in the kidney are still not well understood and requires further study.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (NO.81370701) and the Program for Innovative Research Team at Chongqing University, 2013.

Disclosure

The authors have no conflicts of interest to declare.
References


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Fig. 1 Histomorphological analysis of the kidneys. A-C: H&E staining. (A) No significant
histological abnormalities were observed in the sham group. (B) Tubular dilatation or atrophy, flattened epithelial cells (arrow), interstitial inflammatory cell infiltration, and extracellular matrix deposition were observed in the UUO group. (C) The number and degree of damaged tubules in the CM group were lower than those in the UUO group; and the renal interstitial fibrosis was less in the CM group compared with the UUO group (bar, 50 μm). D-F: PAS staining. (D) The proximal and distal tubules as well as the glomeruli were intact in the sham group. (E) Marked disorganization of the structure of proximal and distal tubules with more cast formation (asterisk) and brush border disruption were observed. (F) The degree of renal tubular injury was significantly reduced in the CM group. G-I: Masson’s trichrome staining. (G) Only a small amount of collagen deposition in the sham group. (H) Extracellular matrix deposition and renal interstitial fibrosis were observed in the UUO group. (I) The renal interstitial fibrosis in the CM group was significantly lower than that in the UUO group. (J) Results of renal tubular histology score. The loss of brush border, tubular dilation and apoptosis/necrosis of tubular cells were assessed. (K) The area of renal interstitial fibrosis in each group. CM transplantation could significantly inhibit renal interstitial fibrosis in UUO kidney. Values are presented as mean ± SD. UUO group is compared with sham and CM groups. Values are statistically significant at *P<0.05; sham group is compared with UUO and CM groups. Values are statically significant at #P<0.05.
Fig. 2 Effects of CM on UUO-induced oxidative stress. (A) Glutathione (GSH), (B) Malondialdehyde (MDA), (C) Reactive oxygen species (ROS) levels in obstructed kidneys at day 14 after UUO were measured. Values are presented as mean ± SD. UUO group is compared with sham and CM groups. Values are statistically significant at *P<0.05; sham group is compared with UUO and CM groups. Values are statically significant at #P<0.05.
Fig. 3 Effects of CM on expression of E-cadherin, TGF-β1, α-SMA, TNF-α, and Collagen-I in each group. Immunohistochemical staining of renal tissue sections by using E-cadherin (A-C), TGF-β1 (D-F), α-SMA (G-I), TNF-α (J-L), and Collagen-I (M-O). (P) Western blot analysis of E-cadherin, TGF-β1, α-SMA, TNF-α, and Collagen-I protein in each group. (Q-U) The relative protein expression levels in kidney tissues. β-actin is used as an inner reference and quantified by using densitometric analysis. Values are presented as mean ± SD. UUO group is compared with sham and CM groups. Values are statistically significant at *P<0.05; sham group is compared with UUO and CM groups. Values are statically significant at #P<0.05.
Fig. 4 Proliferation and apoptosis analysis. (A-C, G): PCNA-positive cells of renal tubules in each group. (A) There was no significant increase in the proliferative activity of renal tubular epithelial cells in the sham group. (B) At 14 days postoperatively, the proliferative activity of renal tubular epithelial cells in the UUO group was significantly increased. (C) The proliferative activity of renal tubular epithelial cells in the CM group was further enhanced compared with UUO group. (D-F, H): TUNEL-positive cells of renal tubules in each group. (D) There was no obvious apoptotic cells in the renal tubular epithelium of the sham group. (E) At 14 days postoperatively, the number of apoptotic cells in renal tubular epithelial cells of UUO group was significantly increased. (F) The number of apoptotic cells of renal tubular epithelial cells in CM group was significantly higher than that in UUO group. Values are presented as mean ± SD. UUO group is compared with sham and CM groups. Values are statistically significant at *P<0.05; sham group is compared with UUO and CM groups. Values are statically significant at #P<0.05.