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The effect of intravenous human adipose-derived stem cells (hADSC) on transforming growth factor β1 (TGF-β1), collagen type 1, and kidney histopathological features in the unilateral ureteropelvic junction obstruction model of wistar rats

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ABSTRACT

Objective: The fibrotic process of kidney resulting in glomerulosclerosis was found in patients with ureteropelvic junction obstruction (UPJO) who underwent renal biopsy during pyeloplasty. Transforming growth factor β1 (TGF-β1) plays a role in collagen accumulation, resulting in fibrosis. Adipose tissue–derived stem cells (ADSCs) have an anti-apoptotic effect on target cells and enhance the kidney function recovery. We will further investigate the use of ADSC in the prevention of kidney fibrosis in the unilateral UPJO model of Wistar rats.

Material and methods: A total of twenty-two 12-week-old Wistar rats were divided into three groups. We made the UPJO models using nylon 6-0 inside the left ureter and tied the ureter with nylon 6-0, creating partial ureteral obstruction. The treatment group was then injected with 1.0×10^6 cells of human ADSC via the tail vein of rats. All rats were euthanized after 2 and 4 weeks of treatment. The left kidney used hematoxylin–eosin for histopathological examination. Statistical analysis using one-way analysis of variance (ANOVA) was done with SPSS version 21.0.

Results: TGF-β1 concentration in the treatment group was significantly lower in the 4th week of observation (p4=0.0001), as well as collagen type 1, which was also significantly lower in the 4th week (p4=0.0001). There was a significant difference in the glomerulus count between the control group and the human ADSC (hADSC) group therapy in week 2 and week 4 (p2=0.0001 and p4=0.026).

Conclusion: Administration of hADSC therapy reduces TGF-β1 and collagen type 1 levels and then improves the histopathological features in the process of renal fibrosis in the UPJO model.

Keywords: Collagen type I; stem cells; TGF-β1; ureteral obstruction.

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Introduction

One of the most common etiologies of hydronephrosis in the pediatric population is the ureteropelvic junction obstruction (UPJO), with an incidence rate around 1 in 1000–1500 people. According to a study in Massachusetts, in 61 pediatric patients with UPJO who underwent a renal biopsy during pyeloplasty, 73% had glomerulosclerosis and 26% had tubular fibrosis.[1] Histological changes in the obstruction process primarily localized in the renal interstitial compartment, including massive tubular dilation, interstitial fibrosis, and reduced kidney size caused by apoptosis. Renal interstitial fibrosis was the most frequent and irreversible pathological process, which may lead to end-stage renal disease. Renal fibrosis was characterized by the destruction of the renal tubules and interstitial capillaries because of an accumulation of extracellular matrix (ECM) through the receptor called transforming growth factor β1 (TGF-β1). This condition results in collagen accumulation with the final result of interstitial fibrosis, epithelial cells apoptosis, and tubular atrophy.[2] Several attempts have been made in preventing kidney fibrosis but still have not been effective in preventing the fibrosis process in UPJO patients.

In recent years, many studies have shown the potential antifibrotic effect of adipose tissue–derived stem cells (ADSCs) in many animal models. ADSC are mesenchymal stem cells that exhibit tissue plasticity and fusiform morphology in vitro. Currently, ADSCs have been used as a therapy for injured organ regeneration in animal models and a few clinical trials. Effects of ADSC were attributed to their extensive secretomes that include interleukins, various growth factors, and other proteins that are suitable inducers of tissue regeneration.[3-6] ADSC also had anti-apoptotic effects on target cells and enhanced recovery of kidney function in ischemic/reperfusion injuries.[7] The result of the study showed that stem cell administration reduced acute kidney damage and inflammation. The administration of stem cells could also inhibit TGF-β1 activation, which played an important role in the process of renal interstitial fibrosis.[2] A study by Li et al. [8] showed that ADSC could reduce collagen type 1 and type 3 and α-Smooth Muscle Actin in vitro. With recent evidences of the antifibrotic effect of ADSC, this study aims to evaluate the potential antifibrotic effect of human ADSC in an experimental model of UPJO.

Material and methods

Human ADSCs isolation and characterization

Human adipose tissue was obtained from the fat of adult male/female subjected to another surgery who had previously signed an informed consent regarding the donation of his/her fat for research. The protocol was approved by the Research and Ethical Committee, which approved the fat obtainment procedure. The tissue was prepared in a sterile petri dish with a 0.075% collagenase type 1 solution (Invitrogen, Grand Island, NY, USA) and phosphate-buffered saline (PBS) containing 2% penicillin/streptomycin (P/S) for tissue digestion, gently shaken for 1 hour at 37°C. Digestion products containing Stromal Vascular Fraction were filtered using a 100 μm nylon mesh and centrifuged at 2000 rpm for 5 minutes. The pellet was washed with PBS, and erythrocytes were lysed. After spinning, all of the collagenase solutions above the pellet were aspirated without disturbing the cells. The pellet was resuspended in 1 mL of lysis buffer, incubated for 10 minutes in ice, and washed with 20 ml of PBS/2% P/S and then centrifuged at 2000 rpm for 5 minutes. The supernatant was aspirated, and the cell pellet was resuspended in a maximum of 3 mL of stromal medium (α-minimum Essential Medium (α-MEM), Mediatech, Herndon, VA, USA) supplemented with 20% fetal bovine serum (FBS), 1% L-glutamine (Mediatech) and 1% P/S, and then the cell suspension was filtered through 70 mm cell strainer. Cells were harvested and seeded until passage 3 to achieve greater expansion. Cell characterization and transplantation were made in this unique batch of cells obtained from one single fat donor in sole isolation.

Main Points:

- Human ADSC therapy can reduce the TGF- β1 concentration in the kidney fibrosis process caused by unilateral UPJO.
- Human ADSC therapy can reduce the collagen type 1 concentration in the kidney fibrosis process caused by unilateral UPJO.
- Human ADSC therapy can repair the glomerulus count in the kidney fibrosis process caused by unilateral UPJO.

In isolation and culture procedures, the percentage of cell vitality was 83.4%. Isolated cells were characterized by flow cytometry assay using the following markers: CD44(+), CD45(–), CD73(+), CD90(+), and CD105(+). A total of 22.5×10^6 cells were obtained and then contributed in α-MEM in 1 cc syringes containing 1.0×10^6 cells for usage. We chose 1×10^6 cells to be injected based on some previous studies that used this formula and approved to have a significant effect as an antifibrotic agent.[9] All tissue harvesting procedures-cell isolation, culture, and characterization-were done in Tissue and Cell Bank of a tertiary hospital in Surabaya and declared suitable for the application.

UPJO Model of Wistar Rats

Twenty-two male Wistar rats (n=10 per group and 2 rats for the negative control group) weighing 250–300 g were separated into three groups. All operations were performed under sterile conditions. The animals were anesthetized with 1-shot ketamine injection (75 mg/kg, intraperitoneally). UPJO was accomplished with the method described by Thornhill et al.[10] Nylon 6-0 suture was inserted through the left ureter (distally from the UPJO) into the kidney. The UPJ was then tied using nylon 6-0, and then the nylon suture was pulled until it came off. Subsequent withdrawing of nylon that resulted in a well-calibrated partial obstruction was used to ensure the partial obstruction.[10]

Only two rats in the control group were used because the control group was not given any treatment, and the aim was only to see normal tissue as a comparison. The use of too many animals was against the principle of animal welfare. After a minimum sample for this study was calculated, four rats were assigned for each group as a minimum sample, with a drop-out factor of 10%. We confirmed 10 rats for each group and 2 rats for the control group. The first group that consisted of 10 male Wistar rats with UPJO model without ADSC therapy were euthanized after 2 and 4 weeks of observation, whereas another group consisting of rats with UPJO model were given 1.0×10^6 cells ADSC injection via the tail vein and euthanized after 2 and 4 weeks of observation. After being euthanized, each rat underwent nephrectomy for TGF-β1 and collagen type I was measured using the enzyme-linked immunosorbent assay (ELISA). Kidney sections were stained by hematoxylin–eosin for histopathological examination and the glomerulus count in ADSC groups and control group were observed by micro-

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scopical examination. The glomerulus count was the objective measurement of the cellular renal function. All animal models used in this study were housed in solid plastic cages, with five rats for each cage. The rats were allowed to eat standard rodent chow and water ad libitum. This study was conducted in the Biochemical and Bioscience Laboratory and had been accepted for ethical clearance from the Ethical Committee. All study objects received treatments according to animal welfare guidelines by William Russel and Rex Burch. For statistical analysis, the groups were compared with one-way analysis of variance (ANOVA). Values 0.05 were considered statistically significant. Statistical analysis was conducted using IBM Statistical Package for the Social Sciences (IBM SPSS Corp.; Armonk, NY, USA) version 21.0.

Results

The study was conducted on 22 male Wistar rats, with 2 rats in group I (negative control group), 10 rats in group II (partial unilateral UPJO model rats without therapy sacrificed in the 2nd week and 4th week), and 10 rats in group III (partial unilateral UPJO model rats without therapy sacrificed in the 2nd week and 4th week). All rats survived to live until the end of the study.

Table 1 and Figure 1 show the mean value of TGF-β1 concentrations in the sham and therapy groups in weeks 2 and 4 based on ELISA. The mean of TGF-β1 in the sham group was 7.33±1.13 in week 2 and 14.53±0.32 in week 4, and in the therapy group, the mean was 7.98±0.77 and 11.12±1.24 in week 2 and week 4, respectively. Statistical analysis of the comparison of TGF-β1 concentrations in the two groups based on the time of observation in a parametric test was done using ANOVA. Before the analysis, a variance homogeneity test was performed using the Levene test. Levene test results for the variance homogeneity of week 2 and week 4: p2=0.398 and p4=0.076 (0.0.5). The mean TGF-β1 concentration in rat kidney tissue showed a significant difference between the TGF-β1 concentration in the sham group and the group given ADSC therapy in week 4 (p=0.0001). There was no increase in TGF-β1 concentration in the control group. In the therapy group, the TGF-β1 concentration tend to increase in week 2 and week 4, but it is seen on the graph that the tendency to increase is lower than the sham group.

Table 2 shows an increase in the concentration of collagen type 1 in the 2nd and 4th weeks of observation in the sham group. In the therapy group, the mean concentration of collagen type 1 in week 2 was slightly higher than the sham group, which was 1.7±0.09 and 1.69±0.03, respectively, and became lower in week 4 (2.58±0.16 and 3.15±0.18). Figure 2 shows that over time, there is a tendency for changes in the collagen type 1 concentration in both groups. Before the analysis, a variance homogeneity test was performed using the Levene test. Levene test results for the homogeneity of variance in the 2nd and 4th weeks: p2=0.100 and p4=0.704 (0.05). The mean concentration of collagen type 1 in rat kidney tissue showed a significant difference between the collagen type 1 concentration in the treatment control group and the group given ADSC therapy in week 4 (p=0.001).

Table 3 shows that there was a glomerular change in the 2nd and 4th weeks of observation. In both groups, the number of glomerulus tend to decrease over time. From Figure 3, it can be seen that over time, there was a tendency for changes in the number of glomerulus, but in the human ADSC (hADSC) therapy group, the number of glomerulus was still higher than the control group. The mean glomerulus count in the sham group was 32.4±1.67 in week 2 and 26.8±8.29 in week 4. In the therapy group, the mean count was 53.00±7.78 in week 2 and 38.0±3.94 in week 4. Levene test results for the variance homogeneity of the 2nd and 4th weeks were

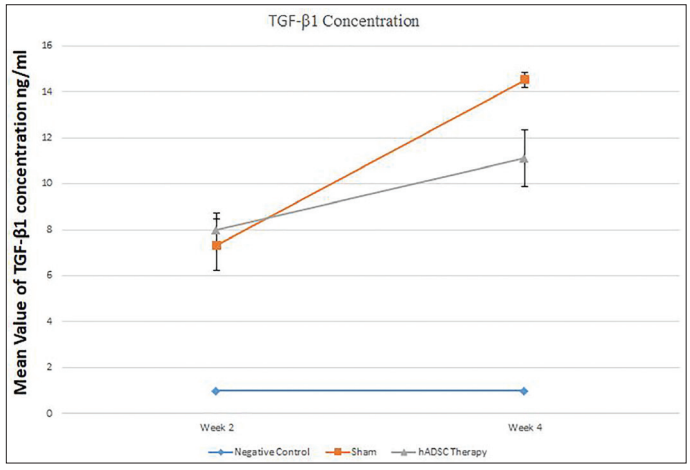

Figure 1. TGF-β1 concentration

Table 1. TGF-β1 concentration between sham group and ADSC group in rat kidney by ELISA

Day of observation		Sham group	ADSC therapy group	p
Week 2	Amount (n)	5	5	0.315
	Mean (ng/mL)	7.33±1.13	7.984763±0.77	
Week 4	Amount (n)	5	5	0.0001
	Mean (ng/mL)	14.53±0.32	11.12109±1.24	

ADSC: adipose-derived stem cells; ELISA: enzyme-linked immunosorbent assay

Table 2. Collagen type I concentration between sham group and ADSC group in rat kidney by ELISA

Day of observation		Sham group	ADSC therapy group	p
Week 2	Amount (n)	5	5	0.833
	Mean (ng/mL)	1.69±0.03	1.70±0.09	
Week 4	Amount (n)	5	5	0.001
	Mean (ng/mL)	3.15±0.18	2.58±0.16	

ADSC: adipose-derived stem cells; ELISA: enzyme-linked immunosorbent assay.

Table 3. Glomerular number counting between sham group and ADSC group in rat kidney

Day of observation		Sham group	ADSC therapy group	p
Week 2	Amount (n)	5	5	0.0001
	Mean (glomerular unit)	32.4±1.67	53±7.78	
Week 4	Amount (n)	5	5	0.026
	Mean (glomerular unit)	26.8±8.29	38±3.94	

ADSC: adipose-derived stem cells

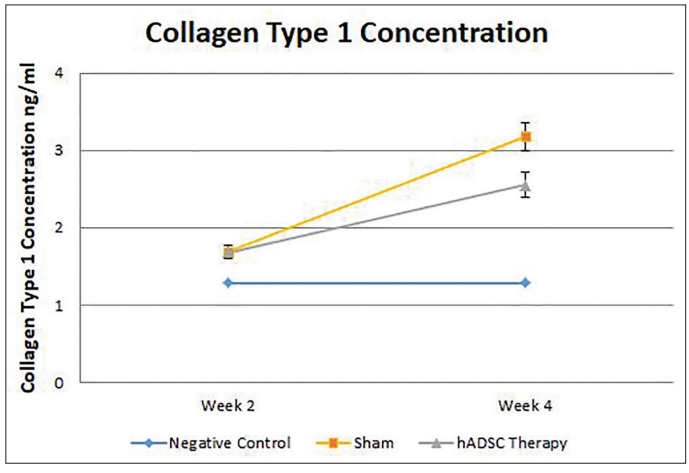

Figure 2. Collagen type 1 concentration

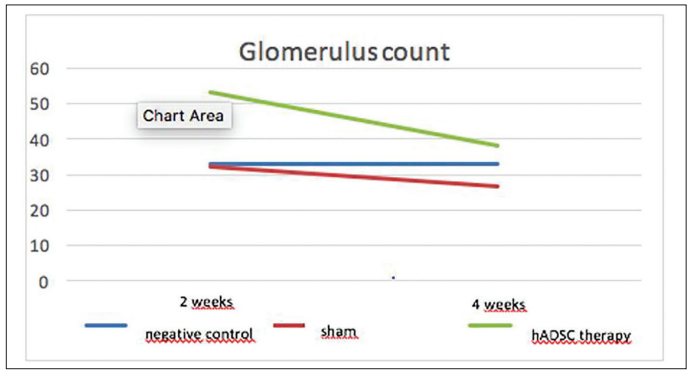

Figure 3. Glomerulus count

p2=0.704 and p4=0.111 (0.05). The mean number of glomerular counts in the ADSC therapy group was significantly higher than the sham group in week 2 (p=0.0001) and week 4 (p=0.026). Figure 4 shows comparation of histopathological feature between control group, sham 2 weeks and 4 weeks observation group, and hADSC therapy 2 weeks and 4 weeks observation group.

Discussion

Urinary tract obstruction is a clinical problem that can occur at any age and lead to kidney damage. One form of urinary tract obstruction is UPJO, which can be caused by congenital abnormalities, benign lesions such as fibroepithelial polyps, urinary tract stones, urothelial malignancies, and postoperative or post-inflammatory scars.

Histological changes associated with the obstruction are usually localized in the renal interstitial compartment and can cause massive tubular dilation, progressive interstitial fibrosis, and loss of kidney mass because of apoptotic cell death.[11] Tubulointerstitial fibrosis is the main pathological component of obstructive kidney injury. The process of fibrosis begins with an increase in inflammatory cell infiltration into the renal interstitial compartment. Macrophage infiltration documented 4 hours after the onset of kidney obstruction and recruitment of macrophages into the interstitial space (like other inflammatory cells) seems to be mediated by chemokine production. All types of kidney cells can express chemokines in response to immunological, toxic, ischemic, or mechanical injury, and the interaction of chemokines with specific receptors expressed on immune cells (chemokine receptors) facilitates the migration of leukocytes and macrophages throughout the endothelium. Once these inflammatory cells fill the interstitium, they begin to elaborate various proinflammatory cytokines and growth factors that contribute to

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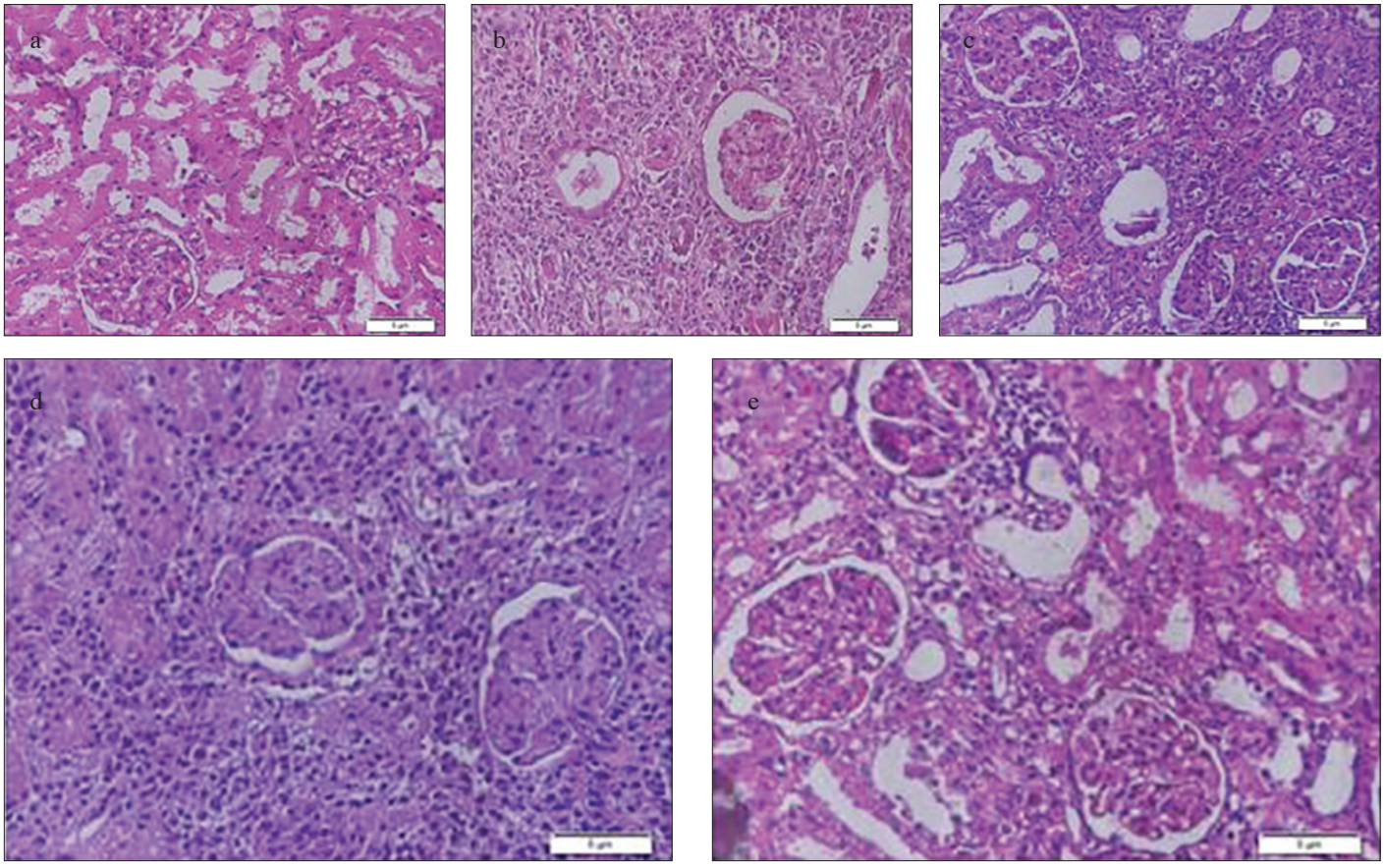

Figure 4. a-e. Kidney histopathology features. (a) normal rat kidney; (b) sham 2 weeks observation; (c) sham 4 weeks observation; (d) hADSC therapy 2 weeks observation; (e) hADSC therapy 4 weeks observation

kidney injury, including tumor necrosis factor-α (TNF-α) and TGF-β1.[11,12] TGF-β1 has long been considered one of the most important mediators of kidney injury caused by obstruction.[13,14] Renal expression of TGF-β1 increases progressively after the onset of obstruction, and evidence shows that TGF-β1 is the main regulator of fibrosis through electromagnetic transients (EMT) simulation and fibroblast proliferation, ECM synthesis, and simultaneous inhibition of collagenase and matrix metalloproteinase (MMPs).[13,15] Excessive expression of TGF-β1 will stimulate fibroblasts to produce collagen type 1 and collagen type 3, resulting in collagen deposits in the renal ECM.[2,9]

In this study, surgery was performed to make partial obstruction of UPJO with the method by Thornhill et al.[10] TGF-β1 and collagen type 1 concentration were found to be higher in the intervention group than in the control group. Short-term acute obstruction was found to be able to cause the same effect as chronic obstruction, with apoptosis and necrosis as the main mechanisms involved in obstructive uropathy. Seven days after partial unilateral ureteral obstruction, there was an increase in tubular cell apoptosis in kidney. After 14 days, there was an increase in cellular proliferation, resulting in tubular atrophy and interstitial fibrosis. From 21 to 42 weeks, the glomerular number was reduced.[16]

In this study, we used ADSC from human adipose tissue because of various reasons: 1) Abundant source of cells from liposuction aspirate. If ADSC from rats was used, several rats have to be sacrificed to get enough adipose tissue for ADSC isolation. 2) The aim to use human ADSC in human patients in the future. Human ADSC was used in rats first because the human ADSC will not express major histocompatibility complex (MHC) class-II, so it will not create a xenogenic immunologic reaction. 3) There is a study comparing the human and rats ADSCs, with 92% (401 types of protein) expressed protein similarity. The result showed no induced immune response.[17]

Adipose-derived stem cells have the antifibrotic ability by suppressing profibrotic genes such as COL1A1, TGF-β1, connective tissue growth factor (CTGF), and actin alpha 2 (ACTA2) and produce various secretions such as vascular endothelial growth factor, hepatocyte growth factor, granulocyte-macrophage colony stimulating factor, basic fibroblast growth factor, brain-derived neurotrophic factor, and insulin growth factor-1

and interleukins such as IL-6, IL-7, IL-8, IL-11, and IL-10, which contribute to restore the microenvironment in the tissue occupied by ADSC, thereby inhibiting the formation of fibrosis tissue by reducing TGF-β1 and the production of collagen type 1 and type 3, which are the main constituents in fibrosis tissue.[17]

Studies by Spiekman showed that ADSC could inhibit fibrotic remodeling by suppressing myofibroblast formation and increasing ECM remodeling. In living things, fibroblast differentiates into myofibroblasts under the influence of TGF-β1. The main features of myofibroblasts are proliferation, contraction, and increased matrix production. The findings of the study stated ADSCs were able to restore fibroblast proliferation to its original level even after stimulation by TGF-β1. ADSC could reduce the expression of 22 alpha-smooth muscle protein (SM22α) in fibroblasts, even after stimulation with TGF-β1, ADSC could reduce dermal fibroblast contractions.[18] Zhang et al.[18] confirm that TGF-β1 could also induce chemotaxis in MSC and increase the TGF-β1 concentration. TGF-β1 to MSC migration is dose-dependent. Research by Zhang et al.[19] in 2018 showed that increasing TGF-β1 receptors on cell membranes would increase ADSC sensitivity to TGF-β1 and accelerated ADSC differentiation into cardiomyocytes by accelerating Smad2/3.57 phosphorylation.

This research was conducted in vivo on experimental animals, which were Wistar rats. It was known that ADSC therapy in mouse models with partial obstruction of the unilateral UPJO caused an increase in TGF-β1 concentrations. Despite this, the improvement in the models that were treated with ADSC was lower than sham. So, the administration of ADSC in the model was considered to reduce TGF-β1, which triggered fibrosis. This was in line with the research conducted by Rivera-Valdes et al.[20] that showed how ADSC administration improved kidney function and decreased fibrosis in the study model.

In fibrosis tissue, fibroblasts differentiate into myofibroblasts. Myofibroblasts produce ECM, which is characterized by large amounts of collagen type 1. Li et al. showed that ADSC caused inhibition of collagen type 1 expression in hypertrophic wound tissue in vitro. Adipose-derived stem cells could also suppress collagen deposits in the matrix tissue and correct fibrosis in the ex vivo matrix. The study also showed the antifibrotic effect of ADSC by regulating the p38/mitogen activated protein kinase (MAPK) pathway, thereby reducing the expression of collagen type 1 and improved fibrotic tissue.[20,21]

In this study, it was found that ADSC administration could reduce levels of collagen type 1 in the process of renal fibrosis because of partial obstruction of the unilateral UPJO. This study showed that over time, there was a tendency for changes in collagen type 1 concentrations in both groups. Besides, there was a significant difference between the concentration of collagen type 1 in the treatment control group and the group given ADSC therapy at 4th week of observation ($p<0.05$).

This study also found that the ADSC administration was influential after the 4th week. This was probably because the antifibrotic effect of ADSC depended on the ADSC dose given according to Haldar et al.[22] research. Also, the ADSC administration method might influence the ADSC antifibrotic effect. Siregar et al.[23] found that intracorporal administration of ADSC in the priapism model of Wistar rats could reduce TGF-β1 and collagen type 1 levels since week 2 when compared with sham.

In this study, it was also known that ADSC administration improved histopathologic features of fibrosis because of obstruction of the unilateral UPJO. We suggested this procedure before surgery because from the previous study, ADSC was known to be able to prevent the fibrotic process in obstructive kidney.

These results were consistent with the research conducted by Donizetti-Oliveira et al.[21], which stated that in the kidneys of animals experiencing hypoxia or ischemia ADSC, administration could decrease the number of areas experiencing hypoxia. Besides, a picture of decreased fibrosis area was obtained after treatment for 6 and 10 weeks in animal models. Donizetti-Oliveira also stated that ADSC therapy could cause developmental stoppages or even reversal of the fibrotic process by reducing inflammation regulation through systemic administration. In Zhang et al.[7] study, it was found that the administration of ADSC to the rat kidney model by ischemia could restore the histological picture of the rat's kidney to normal control.

From this study, we can conclude that hADSC administration could affect the lowering of TGF-β1 and collagen type 1 level. hADSC administration could also improve histopathological features because of partial unilateral UPJO. Further investigation is needed to study ADSC administration in humans. This therapy was considered as adjunctive therapy in UPJO.

Ethics Committee Approval: This study has been approved by the Research and Ethical Committee with ethical number No. 1127-KEP-UB and had been performed in accordance with the ethical standard laid down in the 1964 Declaration of Helsinki and its amendments.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.S., B.S.N., M.I.K.; Design – S.S., B.S.N., M.I.K.; Supervision – S.S., B.S.N.; Resources – S.S., B.S.N.; Materials – S.S., B.S.N.; Data Collection and/or Processing – M.I.K.;

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Analysis and/or Interpretation – M.I.K.; Literature Search – M.I.K.; Writing Manuscript – S.S., B.S.N., M.I.K.; Critical Review – S.S., B.S.N.

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