Am J Transl Res 2016;8(12):5653-5658
www.ajtr.org /ISSN:1943-8141/AJTR0037057

*Original Article*

# Combined thermosensitive in situ gel with AMD3100 in sutureless technique improves the survival and function of kidney transplants in mice

Nengwang Yu[1,2*], Shuai Fu[3*], Junwen Hao[2], Aimin Zhang[2], Zhihou Fu[4]

*[1]Department of Urology, Shandong University Qilu Hospital, Jinan 250012, China; [2]Department of Urology, General Hospital of Jinan Military Command, Jinan 250031, China; [3]Shandong Cancer Hospital & Institute, Shandong Academy of Medical Sciences, Jinan 250117, China; [4]Department of Orthopedics, General Hospital of Jinan Military Command, Jinan 250031, China. [*]Equal contributors.*

Received August 1, 2016; Accepted December 2, 2016; Epub December 15, 2016; Published December 30, 2016

**Abstract:** The mouse is an optimal animal model for kidney transplantation. Recent reports suggest that application of poloxamer 407, a thermosensitive in situ gel, during the sutureless technique significantly increases animal survival, compared to traditional methods. However, further improvement of this technology is greatly needed but remains unexplored. Here, we detected significant inflammation at the region of ureter anastomosis, after kidney transplantation using poloxamer 407. Since chemokines play a pivotal role during inflammation, we implanted an Alzet osmotic pump that gradually releases AMD3100 (a specific inhibitor of the binding of stromal cell-derived factor 1 (SDF-1) to its receptor, CXCR4) at the site of ureter anastomosis in mice that had undergone kidney transplantation. We found that AMD3100 significantly reduced local inflammation, significantly improved animal survival after kidney transplantation, and significantly improved kidney function. Together, these data suggest that inhibition of chemokine signaling at the site of ureter anastomosis may substantially improve animal survival after kidney transplantation through suppression of suturing-related inflammation.

**Keywords:** Kidney transplantation, thermosensitive in situ gel poloxamer 407, inflammation, stromal cell-derived factor 1 (SDF-1), CXCR4, AM3100, ureter anastomosis

## Introduction

Kidney transplants in the mouse model are a very valuable tool for studying transplantation-associated biology and immunology [1]. However, the technical complexity of this surgery in mice, especially the ureter anastomosis, leads to high mortality rates, which block the procedure's widespread application [2]. Recently, some alternatives to sutures have been proposed and examined; specifically, a new method of suturing that uses poloxamer 407, a US Food and Drug Administration (FDA)-approved thermosensitive polymer, was found to temporarily yet effectively maintain an open lumen for ureter anastomosis. The use of poloxamer 407 has been shown to improve animal survival after complex surgeries involving anastomosis [3]. However, optimization of this technique is still needed.

Sutures and sutureless gel both create local inflammation [4-6], which has been implicated in both transplant dysfunction and reduced survival rates in animals. Among the chemokines that regulate initiation and progression of inflammation, stromal cell-derived factor 1 (SDF-1) is the most important. SDF-1 is also known as C-X-C motif chemokine 12 (CXCL12), which is key for activating lymphocytes and macrophages to initiate inflammation [7]. SDF-1, which is normally produced and secreted at the site of injury, recruits inflammatory cells by targeting CXCR4, which is expressed on the cell surface [8]. Recently, the SDF-1/CXCR4 axis has been described as a retention signal for M2 macrophages [9, 10]. Nevertheless, a role for SDF-1 in the development of sutureless gel-associated inflammation has not been reported.

Here, we report that significant inflammation was detected in mice at the region of ureter anastomosis, after kidney transplantation with poloxamer 407. We implanted an Alzet osmotic pump that gradually releases AMD3100, a spe-

cific inhibitor for the binding of SDF-1 to its receptor CXCR4, at the site of ureter anastomosis in mice that had undergone kidney transplantation. We found that AMD3100 significantly reduced local inflammation, significantly improved animal survival after kidney transplantation, and significantly improved kidney function.

## Materials and methods

### *Mouse procedures*

All mouse experiments were approved by the Animal Research and Care Committee at the General Hospital of Jinan Military Command. Forty C57/BL6 mice were purchased from Jackson Labs (Bar Harbor, ME, USA). Male mice at 10 weeks of age were subjected to kidney transplantation using kidneys from isogeneic mouse donors. To inhibit SDF-1/CXCR4 interaction, 2 mg of AMD3100 (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in PBS and placed in mini-osmotic pumps (Alzet Osmotic Pumps, Cupertino, CA, USA), which were then implanted at the site of ureter anastomosis in 20 mice, at the time of kidney transplantation. Mini-pumps containing saline were implanted into another 20 mice as a control. After 2 weeks, 5 mice from each group were sacrificed for analysis of local inflammation. The other 15 mice in each group were kept for another 6 weeks (8 weeks in total following kidney transplantation and AMD3100/control pump implantation) for evaluation of survival and kidney function.

### *Flow cytometry*

The tissue digests were analyzed for relative enrichment of CD45+ cells by flow cytometry, using a PE-cy7-conjugated rat anti-mouse CD45 antibody (Becton-Dickinson Biosciences, San Jose, CA, USA).

### *Immunohistochemistry (IHC)*

The mouse tissue (kidney or site of ureter anastomosis) was fixed in 4% formalin for 6 hours, cryo-protected in 30% sucrose overnight, and then sectioned at 6 μm. IHC was then performed, followed by counterstaining with Hematoxylin. The primary antibody for IHC was polyclonal rat anti-CD45 (DAKO, Carpinteria, CA, USA). DAPI was used to stain the nucleus. For quantification, 5 random fields were quantified in each slide, and in each animal, at least 5 slides that were 100 μM away from each other were counted. Quantification was performed for 15 mice in each condition.

### *Kidney morphology*

To assess glomerular injury, renal tissues were subjected to periodic acid Schiff (PAS) staining. Glomerulosclerosis (GS) was evaluated by determining the percentage of glomeruli exhibiting sclerotic lesions (% GS). For each mouse, 100 consecutive glomeruli were examined. Lesion scores related to the glomerular and mesangial area were graded based on the following criteria: 0=preservation of normal architecture, 1=5-15% glomerular expansion or mesangial expansion and PAS positivity, 2= 15-30% glomerular expansion or mesangial expansion and PAS positivity, 3=30-50% glomerular expansion or mesangial expansion and PAS positivity, and 4≥50% g lomerular expansion or mesangial expansion and PAS positivity.

### *Renal function*

Blood samples were centrifuged at 2000 g for 10 min, and serum was collected for measurement of blood urea nitrogen (BUN) with a BUN measurement kit (Sigma-Aldrich, St. Louis, MO, USA). Urine samples were centrifuged in the same way to remove any suspended particles, and the supernatant was used to detect 24-hour urine albumin protein with an albumin mouse ELISA Kit (Abcam, Cambridge, MA, USA). Creatinine clearance was measured with a creatinine companion kit (Exocell inc., Philadelphia, PA, USA), calculated as urinary creatinine X urine volume/serum creatinine, and expressed as milliliters per minute.

### *Statistical analysis*

All statistical analyses were carried out using the SPSS 17.0 statistical software package. All values are depicted as mean ± standard deviation and are considered significant if $P<0.05$. Mice survival was calculated using Kaplan-Meier analysis. All data were statistically analyzed using one-way ANOVA with a Bonferroni correction, followed by Fisher's Exact Text to compare two groups.

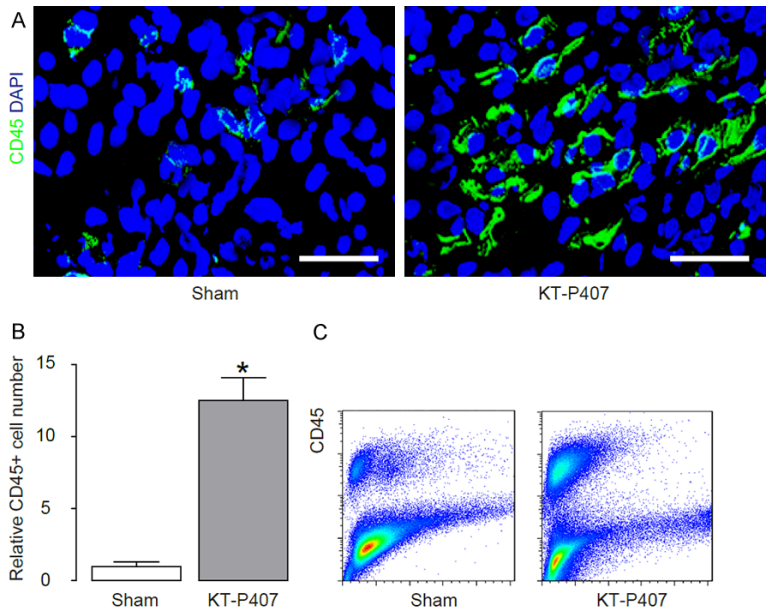


**Figure 1.** Significant inflammation is detected at the region of ureter anastomosis by poloxamer 407 after kidney transplantation. (A) Representative CD45 at the site of ureter anastomosis in control (Sham; sham operated, no kidney transplantation and ureter anastomosis performed) and experimental mice (KT-P407; using poloxamer 407 in kidney transplantation) 2 weeks after surgery. (B, C) Flow cytometry for CD45 of tissue cell digests at the region of ureter anastomosis in Sham and KT-P407 mice 2 weeks after surgery, shown by quantification (B) and by representative flow charts (C). *P<0.05. N=5. Scale bars are 20 μm.

## Results

*Significant inflammation is detected at the region of ureter anastomosis by poloxamer 407 after kidney transplantation*

We examined the inflammation at the site of ureter anastomosis by staining the tissue for CD45, a pan-leukocyte marker. We found that, compared to control (sham operated, no kidney transplantation and ureter anastomosis performed), more CD45+ cells were detected at the region of ureter anastomosis by poloxamer 407 after kidney transplantation (**Figure 1A**). To quantify this finding, we isolated tissue at the region of ureter anastomosis and dissociated it into single cells for flow cytometry analysis using CD45. We found that, compared to control (sham operated, no kidney transplantation and ureter anastomosis performed), significantly more CD45+ cells were detected at the region of ureter anastomosis by poloxamer 407 after kidney transplantation, shown by quantification (**Figure 1B**), and by representative flow charts (**Figure 1C**). Thus, ureter anastomosis by poloxamer 407 after kidney transplantation also creates inflammation.

*Experimental suppression of inflammation improves mouse survival after kidney transplantation*

Since chemokines play a pivotal role in inflammation, we implanted an Alzet osmotic pump that gradually releases AMD3100 (a specific inhibitor for the binding of stromal cell-derived factor 1 (SDF-1) and its receptor CXCR4) at the site of ureter anastomosis in mice that had undergone kidney transplantation. Control mice that similarly underwent kidney transplantation received Mini-pumps containing saline. After 2 weeks, a subset of mice from each group were sacrificed for analysis of local inflammation. The remaining mice in each group were kept for another 6 weeks for evaluation of survival and kidney function (**Figure 2A**). We found that AMD3100 administration significantly improved animal survival after kidney transplantation (**Figure 2B**).

*Experimental suppression of inflammation reduces damage to the kidney transplant*

Next, we examined the morphology of the mouse kidney transplants after PAS staining. Compared to control saline-treated mice, the mice that were administered AMD3100 exhibited significantly reduced glomerular fibrosis, shown by lesion score quantification (**Figure 3A**), and by representative images (**Figure 3B**). Thus, experimental suppression of inflammation reduces damage to the kidney transplant.

*Experimental suppression of inflammation improves kidney transplant function*

Next, we examined the function of the kidney transplants. Compared to control saline-treated mice, mice that were administered AMD3100 exhibited significantly reduced serum blood

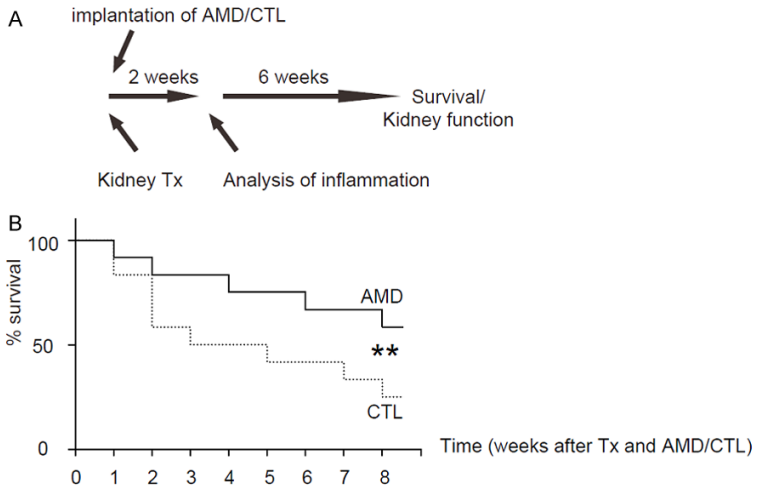


**Figure 2.** Experimental suppression of inflammation improves mouse survival after kidney transplantation. A. Schematic of the experiment: An Alzet osmotic pump that gradually releases AMD3100 was implanted into mice at the time of kidney transplantation (AMD). The control mice that similarly underwent kidney transplantation received Mini-pumps containing saline (CTL). After 2 weeks, a subset of mice from each group were sacrificed for analysis of local inflammation. The other mice in each group were kept for another 6 weeks for evaluation of survival and kidney function. B. Mice survival after kidney transplantation. **P<0.01. N=30. Tx: transplantation.

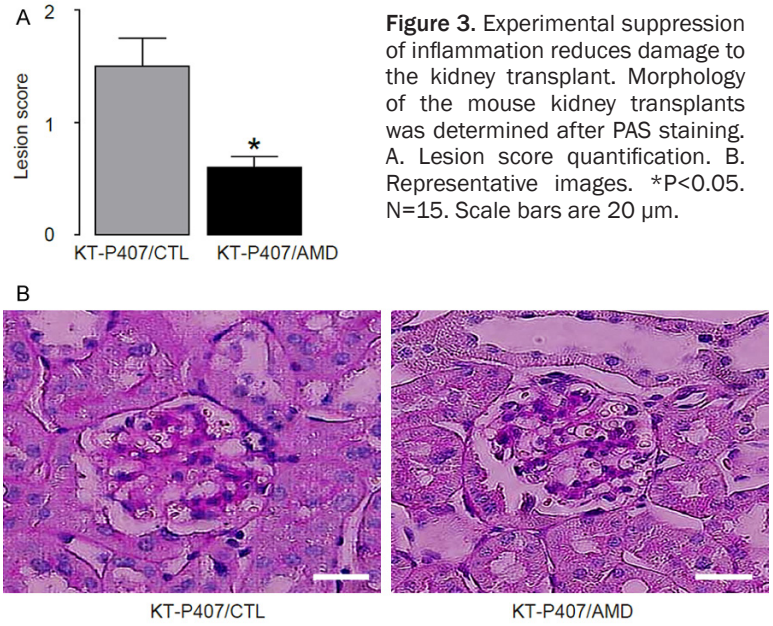


**Figure 3.** Experimental suppression of inflammation reduces damage to the kidney transplant. Morphology of the mouse kidney transplants was determined after PAS staining. A. Lesion score quantification. B. Representative images. *P<0.05. N=15. Scale bars are 20 μm.

urea nitrogen (BUN) (**Figure 4A**), significantly increased creatinine clearance rate (CCR) (**Figure 4B**), and significantly reduced urinary protein excretion (**Figure 4C**). Thus, experimental suppression of inflammation improves kidney transplant function.

*AMD3100 reduces local inflammation*

As a control, we examined inflammation at the site of ureter anastomosis by staining the tissue for CD45, two weeks after AMD3100/saline administration. We found that, compared to control (kidney transplantation, saline pump implanted), fewer CD45+ cells were detected at the region of ureter anastomosis by poloxamer 407 after kidney transplantation in mice that received AMD3100 (**Figure 5A**). To quantify this finding, we isolated tissue at the region of ureter anastomosis and dissociated it into single cells for flow cytometry analysis using CD45. We found that, compared to control, significantly fewer CD45+ cells were detected at the region of ureter anastomosis by poloxamer 407 after kidney transplantation in mice that received AMD3100, shown by quantification (**Figure 5B**), and by representative flow charts (**Figure 5C**). Thus, AMD3100 indeed reduces local inflammation.

## Discussion

The mouse is an important model for studying kidney transplantation, both because of its relative inexpensiveness and the availability of transgenic mice [1]. However, the mouse ureter is very small, and traditional suturing methods for ureter anastomosis are technically demanding. Even for those with extensive training, mouse kidney transplantation leads to significant mortality [2]. Therefore, alternative suturing methods or sutureless technology are critical for improving the efficacy of kidney transplantation in mice.

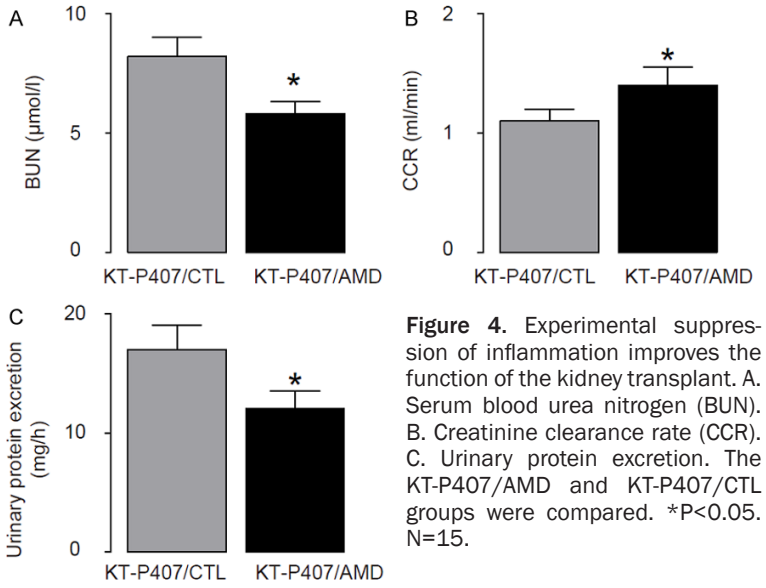


**Figure 4.** Experimental suppression of inflammation improves the function of the kidney transplant. A. Serum blood urea nitrogen (BUN). B. Creatinine clearance rate (CCR). C. Urinary protein excretion. The KT-P407/AMD and KT-P407/CTL groups were compared. *P<0.05. N=15.

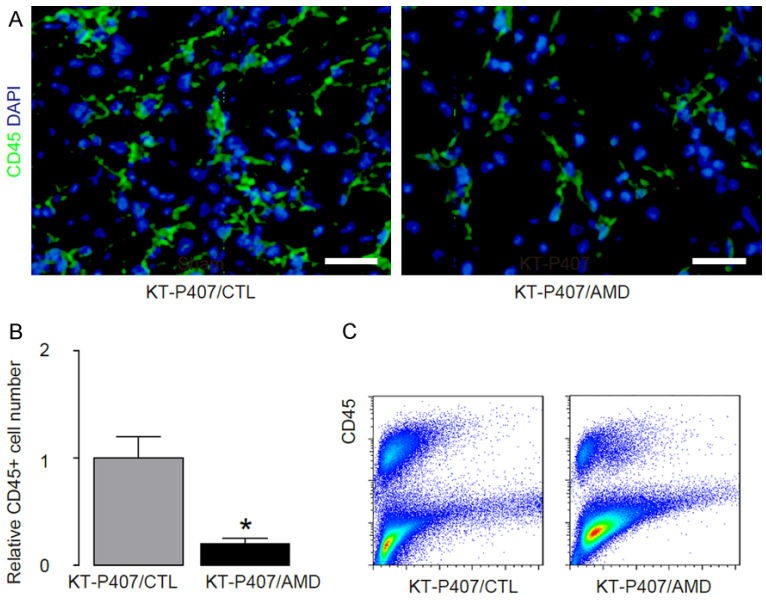


**Figure 5.** AMD3100 reduces local inflammation. (A) Representative CD45 at the site of ureter anastomosis in KT-P407/CTL and KT-P407/AMD groups 2 weeks after surgery. (B, C) Flow cytometry for CD45 of tissue cell digests at the region of ureter anastomosis in KT-P407/CTL and KT-P407/AMD mice 2 weeks after surgery, shown by quantification (B) and by representative flow charts (C). *P<0.05. N=5. Scale bars are 30 μm.

The recent application of a new sutureless method with poloxamer 407 begins to achieve this goal [3]. Application of this technology seemed to significantly improve animal survival after anastomosis, mainly through reduction in tissue damage. However, the application of sutureless materials may create local inflammation, which not only impairs the recovery from surgery, but also promotes host-versus-graft rejection [4-6]. Thus, we hypothesize that experimental control of poloxamer 407-associated inflammation may further improve outcomes in kidney transplantation in mice.

In order to suppress local inflammation, we used an AMD3100 pump system, taking advantage of its effective inhibition of SDF-1/CXCR4 axis, which plays a pivotal role in the development of inflammation [11, 12]. We detected significant inflammation at the region of anastomosis of the mouse ureter after kidney transplantation with poloxamer 407, which confirms our hypothesis that poloxamer 407 may create inflammation. Moreover, we found that AMD3100 significantly reduced local inflammation, as evidenced by examination of CD45+ cell infiltration locally by both immunohistochemistry and flow cytometry. In addition, suppression of local inflammation significantly improved both animal survival and kidney function after kidney transplantation. These findings may result from reduced inflammatory damage to the kidney transplant and from reduced adverse effects of inflammation on the graft.

Together, our data suggest that application of combined thermosensitive in situ gel with AMD3100 in the sutureless technique, may improve the survival and function of kidney transplants in mice, through suppression of inflammation. This study may be clinically translatable to humans, and should be further examined in future studies.

## Acknowledgements

The study is supported by the National Natural Science Foundation of China (No. 81300624) and the China Postdoctoral Science Foundation (No. 2013T60953).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Nengwang Yu, Department of Urology, Shandong University Qilu Hospital, 107 Wenhua Xi Road, Jinan 250012, China. Tel: +8653151665311; Fax: +8653151666223; E-mail: yunengwang@hotmail.com; Zhihou Fu, Department of Orthopedics, General Hospital of Jinan Military Command, 25 Shifan Road, Jinan 250031, China. Tel: +8653151666021; Fax: +8653151666223; E-mail: ynw533@yeah.net

## References

[1] Ge F and Gong W. Strategies for successfully establishing a kidney transplant in a mouse model. Exp Clin Transplant 2011; 9: 287-294.

[2] Plenter R, Jain S, Ruller CM, Nydam TL and Jani AH. Murine kidney transplant technique. J Vis Exp 2015; e52848.

[3] Chang EI, Galvez MG, Glotzbach JP, Hamou CD, El-ftesi S, Rappleye CT, Sommer KM, Rajadas J, Abilez OJ, Fuller GG, Longaker MT and Gurtner GC. Vascular anastomosis using controlled phase transitions in poloxamer gels. Nat Med 2011; 17: 1147-1152.

[4] Gupta A, Broin PO, Bao Y, Pullman J, Kamal L, Ajaimy M, Lubetzky M, Colovai A, Schwartz D, de Boccardo G, Golden A and Akalin E. Clinical and molecular significance of microvascular inflammation in transplant kidney biopsies. Kidney Int 2016; 89: 217-225.

[5] Sellares J, de Freitas DG, Mengel M, Sis B, Hidalgo LG, Matas AJ, Kaplan B and Halloran PF. Inflammation lesions in kidney transplant biopsies: association with survival is due to the underlying diseases. Am J Transplant 2011; 11: 489-499.

[6] Molnar MZ, Czira ME, Rudas A, Ujszaszi A, Lindner A, Fornadi K, Kiss I, Remport A, Novak M, Kennedy SH, Rosivall L, Kovesdy CP and Mucsi I. Association of the malnutrition-inflammation score with clinical outcomes in kidney transplant recipients. Am J Kidney Dis 2011; 58: 101-108.

[7] Fan Y, Xu LL, Shi CY, Wei W, Wang DS and Cai DF. MicroRNA-454 regulates stromal cell derived factor-1 in the control of the growth of pancreatic ductal adenocarcinoma. Sci Rep 2016; 6: 22793.

[8] Chen D, Xia Y, Zuo K, Wang Y, Zhang S, Kuang D, Duan Y, Zhao X and Wang G. Crosstalk between SDF-1/CXCR4 and SDF-1/CXCR7 in cardiac stem cell migration. Sci Rep 2015; 5: 16813.

[9] Grunewald M, Avraham I, Dor Y, Bachar-Lustig E, Itin A, Jung S, Chimenti S, Landsman L, Abramovitch R and Keshet E. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell 2006; 124: 175-189.

[10] Cao X, Han ZB, Zhao H and Liu Q. Transplantation of mesenchymal stem cells recruits trophic macrophages to induce pancreatic beta cell regeneration in diabetic mice. Int J Biochem Cell Biol 2014; 53: 372-379.

[11] Ueno T, Nakashima A, Doi S, Kawamoto T, Honda K, Yokoyama Y, Doi T, Higashi Y, Yorioka N, Kato Y, Kohno N and Masaki T. Mesenchymal stem cells ameliorate experimental peritoneal fibrosis by suppressing inflammation and inhibiting TGF-beta1 signaling. Kidney Int 2013; 84: 297-307.

[12] Semedo P, Palasio CG, Oliveira CD, Feitoza CQ, Goncalves GM, Cenedeze MA, Wang PM, Teixeira VP, Reis MA, Pacheco-Silva A and Camara NO. Early modulation of inflammation by mesenchymal stem cell after acute kidney injury. Int Immunopharmacol 2009; 9: 677-682.