The Effects of Indoxyl Sulfate on Human Umbilical Cord-Derived Mesenchymal Stem Cells In Vitro

Wei Wang, Xueyong Liu, Wei Wang, Jinghua Li, Yuanyuan Li, Liping Li, Shaohua Wang, Jianchun Zhang, Youkang Zhang, Haichang Huang

The Kidney Disease Research Center, Jingdong Yumei Kidney Disease Hospital, Beijing, Renal Division, Key Laboratory of Renal Disease, Department of Medicine, Peking University First Hospital, Beijing, China

Key Words
Uremic toxins • Human mesenchymal stem cells • Cellular senescence • CD4⁺ regulatory T cells

Abstract
Background/Aims: Indoxyl sulfate, an important protein-bound uremic toxin, can damage stem cells, thus hampering stem cell-based regenerative medicine approaches targeting chronic kidney diseases (CKD). Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) are thought to have promising clinical application because of their high proliferative potential and ease of isolation than MSCs from other sources. In the present study, we aimed to determine the harmful effects of indoxyl sulfate on the phenotype and functional potential of hUC-MSCs in vitro. Methods: The toxicity and cell viability was examined by Trypan blue exclusion and MTT assay. The cellular surface markers and the percentage of apoptotic cells by Annexin-V/PI staining were analyzed by flow cytometry. Proliferation was evaluated based on cell number counting and Ki-67 immunostaining. Cell senescence was measured using senescence-associated β-Galactosidase activity. The ability to stimulate the development of CD4⁺CD25⁺FoxP3⁺ regulatory T cells was assessed by incubating hUC-MSCs with peripheral blood mononuclear cells from the healthy volunteers. Results: Our results demonstrated that the immunophenotype of hUC-MSCs was not affected by indoxyl sulfate flow cytometry. However, a significant decrease in cell numbers and fraction of Ki-67 positive proliferating cells, along with a significant increase in cellular senescence were detected in hUC-MSCs after exposure to indoxyl sulfate. Additionally, their ability to stimulate CD4⁺CD25⁺FoxP3⁺ regulatory T cell production was compromised when hUC-MSCs were pretreated with indoxyl sulfate. Conclusion: Taken together, our study clearly demonstrated that the molecular alterations and functional incompetence in hUC-MSCs under the challenge of indoxyl sulfate in vitro.
Introduction

Chronic kidney disease (CKD) is associated with inflammation, which may be caused by and can also be a consequence of oxidative stress. Both chronic inflammation and oxidative stress may contribute to the progression of CKD and many of the CKD-associated complications, and lead to significant increase in cardiovascular disease (CVD) and all-cause mortality [1]. One potential cause of oxidative stress and inflammation is retained uremic metabolites, also known as uremic toxins [2]. The subset of toxins emanating from microbial metabolism has been associated with progression of CKD and development of CKD-related complications, including the progression to renal failure, accelerated cardiovascular disease, and uremic bone disease [3, 4]. Two important protein-bound uremic toxins that have been linked to adverse outcomes in CKD patients are indoxyl sulfate and p-cresyl sulfate [5]. Indoxyl sulfate is a protein-bound uremic solute resulting from the bacterial metabolism of dietary tryptophan to indole. Indoxyl sulfate is normally cleared by means of renal proximal tubular secretion; however, in patients with CKD, impaired renal function leads to its accumulation. Bound to serum albumin, indoxyl sulfate is not removed effectively by hemodialysis [6].

Cell-based therapy is a promising clinical approach for several pathological inflammatory conditions, and might represent a novel therapeutic strategy to slow the progression of kidney disease [7-9]. A number of different types of cells from the bone marrow have been tested in animals and in clinical studies for potential use in kidney disease. Amongst all the cells under investigation, mesenchymal stem cells (MSCs) have shown the most promising results till date. Many, and mostly positive, results of studies employing mesenchymal stem cell therapy for treatment of experimental acute kidney injury (AKI) [8, 9] have been reported. However, mesenchymal stem cells have recently received more attention for their potential therapeutic effects in CKD patients [10, 11]. Mesenchymal stem cells can exert their immunomodulatory effects through both contact dependent and contact independent (via soluble factors) mechanisms. Studies in animal models of chronic renal failure have uncovered a unique potential of these cells for improving function and regenerating the damaged kidney via the reduction of disease-related inflammation and fibrosis [12, 13]. Mesenchymal stem cells can be isolated from a variety of tissues. Although human bone marrow-derived MSCs are commonly used in clinical trials, human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) can prove to be a more suitable source of transplantable MSCs due to their high proliferative potential and ease of isolation. hUC-MSCs are intensely studied for therapeutic use since they are more primitive and have a rapidly growing capacity to meet large-scale expansion demands than MSCs from other sources. There is also an unlimited supply of umbilical cord, easy access to the cells, and less ethical concerns [14-16].

Recently, there were several reports investigating the toxic effects of uremic toxins on human bone marrow-derived MSCs [17, 18]. However, little is known about the effects of uremic toxins on human umbilical cord-derived mesenchymal stem cells. Evidences show that the toxic effects of indoxyl sulphate associated prominently with adverse outcomes in CKD, i.e. indoxyl sulphate most intensely induce endothelial ROS production [19]. Furthermore, it has been shown that indoxyl sulfate but not p-cresyl Sulfate is associated with advanced glycation end products in patients on long-term hemodialysis [20]. In the present study, indoxyl sulphate was chosen as one contributor of the two most important protein-bound uremic toxins, and we investigated the deleterious impact of indoxyl sulfate on phenotypic and functional characteristics of hUC-MSCs in vitro.

Materials and Methods

**Ethics**

Human ethics approval was obtained from Clinical Research Ethics Committee of Yumei Kidney Disease Hospital for the use of human tissues. The informed written consent form was approved by the Clinical Research Ethics Committee and signed by the donor before sample collection.
Isolation, culture, and characterizations of human umbilical cord-derived mesenchymal stem cells

The protocol for isolation and culture of human umbilical cord-derived mesenchymal stem cells were adapted from methods described by Mennan et al. [21]. In brief, the umbilical cord samples were collected from full term caesarean section mothers by aseptic collection and cut into pieces of 0.5–1 mm\(^2\). The pieces were placed in T75 flasks for 2 hours and cultured in DMEM (Gibco) with 10% FBS (Gibco). Flasks were incubated with saturated humidity at 37°C containing 5% CO\(_2\), and the medium was changed every 3 days. When the cells grew out and reached 80% confluence, the cord tissues were removed and discarded, while the cells were trypsinized and replated at a density of 1.0 × 10\(^6\) cells per T75 flask; these cells were considered as passage 1. The cells were cultured in 10% FBS DMEM/F12 (50:50) (Gibco). For all experiments, passage 2 and passage 3 were used. The morphology was assessed during the culture.

Flow cytometry analysis

The immunophenotype of cultured hUC-SMCs were determined by flow cytometry FACSCalibur with the human mesenchymal stem cells analysis kit (BD Biosciences): hMSC positive cocktail (FITC-CD90, PerCP-cy5.5-CD105, and APC-CD73), and the PE-hMSC Negative Cocktail (CD11b, CD19, CD34, CD45, and HLA-DR).

To analyze surface antigens after treatment with indoxyl sulfate (Sigma-Aldrich), hUC-MSCs were incubated in 5% FBS DMEM/F12 without (0 µM, control) or with 400 and 800 µM indoxyl sulfate for 4 days. The cells were detached with 0.05% trypsin at 4 days and washed 3 times with phosphate-buffered saline (PBS)/2% fetal calf serum. Cells were stained with the hMSC positive cocktail before analysis. The data was processed using Cell Quest Pro software (BD).

Trypan blue exclusion assay

1 × 10\(^4\) cells/well (triplicate each group) hUC-MSCs were seeded in 24-well culture plates and incubated in 10% FBS DMEM/F12 without or with 400 and 800 µM indoxyl sulfate for 2 and 4 days. Cells were trypsinized and centrifuged at 500g for 5 minutes. The cell pellets were stained with 0.4% trypan blue solution (Gibco) at 1:10 for 1 minute. Cells were counted by hemocytometer under an inverted light microscope Nikon Ts100 at low magnification. We calculated cell viability by the formula: % viable cells = [1.00–(Number of blue cells ÷Number of total cells)] × 100.

MTT colorimetric assay

hUC-MSCs were seeded into 96-well culture plates (6 × 10\(^3\) cells/well) and treated without or with 400 and 800 µM indoxyl sulfate for 12 and 24 h. Medium supernatants were removed, and cells were detected with MTT cell proliferation and cytotoxicity assay kit (Solarbio). Cells were incubated with MTT solution (10µl MTT in 90µl DMEM) for 4 h at 37°C. Formazan was solubilized by 110 µl Formazan solution, and the absorbance (OD) was measured at 490 nm by a microplate reader Multiskan EX ELISA reader (Thermo Fisher).

Cell apoptosis assay

Apoptotic cell death was measured with FITC-Annexin V ant PI double staining apoptotic assay kit (BestBio) by flow cytometry. hUC-MSCs (1 × 10\(^5\) cells per) in 6-well culture plates were treated without or with 400 and 800 µM indoxyl sulfate at different time points. Cells were trypsinized and centrifuged at 500g for 5 minutes. The cell pellets were washed with cold PBS twice. Cells were dispersed in 400 µl binding buffer with 5 µl of Annexin V-FITC at 4°C for 15 minutes in the dark. Afterward, 10 µl of PI was added to stained cells at 4°C for 5 minutes. Cells were submitted to flow cytometry.
Cell number counting

To examine the effect of indoxyl sulfate on the cell growth and proliferation of hUC-MSCs, we monitored the number of cells over time as the assays for cell proliferation. hUC-MSCs were seeded in 48-well culture plates (5 × 10^3 cells/well, triplicate per group) and treated without or with 400 and 800 µM indoxyl sulfate for 2, 4, and 8 days. The cells were trypsinized and collected in 10% FBS DMEM/F12 medium. The number of cells was counted using hemocytometer under a light microscope. Each well was repeated three times and mean value of cells were calculated in each group.

Ki-67 immunostaining assay

Semi-quantitative measurement of Ki-67 positive immunostained cells also was applied to assess the cell proliferation. 1 × 10^4 cells of hUC-MSCs (triplicate per group) were planted on cover slips in 24-well culture plates, and cultured in 5% FBS DMEM/F12 medium without or with 400 and 800 µM indoxyl sulfate for 2, 4, and 8 days. Cells were harvested and fixed by 3.7% formalin for 15 minutes. Then they were washed twice with PBS, permeabilized with 0.5% Triton-PBS for 15 minutes, and washed twice with PBS. Cells were incubated in 1%BSA at room temperature for 30 minutes to block nonspecific binding. Ki-67 immunostaining was carried out using the rabbit polyclonal antibody against Ki-67 (Santa Cruz) at 1:80 dilutions, and incubated overnight at 4°C. Cells were washed twice with PBS and incubated with HRP-conjugated goat anti-rabbit IgG (Santa Cruz) in 1%BSA-PBS at room temperature for 60 minutes. After washing, the cells on the cover slips were developed with the addition 3,3′-Diaminobenzidine (Amresco) solution followed by counterstaining with hematoxylin. The stained slides were then examined by light microscopy to assess the expression of Ki-67. Semi-quantitative measurement of the fraction of Ki-67 positive cells was conducted at random 10 fields in each cover slip under an imaging microscope system Nikon80i at low magnification; three coverslips were counted in each group.

Cellular senescence assessment

Cellular senescence was was detected with senescence β-Galactosidase staining kit (Cell Signaling) following the manufacturer’s instructions. hUC-SMCs were seeded and treated as the same as the above. Cells on the cover slips were harvested and washed three times with PBS, and were fixed with the fixative solution at room temperature for 15 minutes. After washing twice with PBS, cells were incubated with β-galactosidase staining solution. Plates were sealed with parafilm to prevent evaporation and incubated at 37°C overnight. The development of blue color in the cells was checked and slightly washed with water. The percentages of β-gal-positive cells determined by counting 10 random fields under an inverted light microscope Nikon Ts100; three cover slips were counted in each group.

Isolation of peripheral blood mononuclear cells and co-cultured with hUC-MSCs

Healthy volunteer subjects were recruited from the Jingdong Yumei Kidney Disease Hospital. All participants provided informed consent, signed by the donor before sample collection.

Peripheral blood mononuclear cells (PBMCs) from healthy volunteer subjects were obtained from venous anticoagulant blood by using Histopaque-1077 (Sigma-Aldrich) gradient centrifugation and co-cultured with hUC-SMCs in 10% FBS RPMI-1640 GlutaMAX (Gibco) at 37°C in humidified air with 5% CO₂.

Before co-culturing with PBMCs, 4 × 10^4 hUC-MSCs were seeded in 12-well culture plates and were pretreated without or with 400 and 800 µM indoxyl sulfate for 2 days. The isolated PBMCs from healthy volunteers were added to the wells and co-cultured with pretreated hUC-SMCs at 1:5 for 4 days. At the end of co-culture, PBMCs were harvested, and the frequency of CD4^+CD25^+FoxP3^+ regulatory T cells was analyzed according to the method described by Prevosto et al. [22].

Statistics

Data was presented as means ± S.E.M from at least three independent experiments. The significance of differences between comparative groups was analyzed using Student’s t-test and one way-ANOVA. P < 0.05 was considered statistically significant.
Results

Effects of indoxyl sulfate on hUC-MSCs surface markers

Cultured hUC-MSCs displayed the typical fibroblast-like shape and were adherent to the flask surface. The immunophenotype of outgrown cells was confirmed as more than 95% of the cells were positive for CD73, CD90, and CD105 (Fig. 1A). The indoxyl sulfate plasma concentration in patients with chronic kidney disease range from 62.5 µM to 1000 µM [23], and in vitro studies this concentration range from 200 µM to 1000 µM [17, 23-25]. To evaluate the harmful effects of indoxyl sulfate on cultured hUC-MSCs, we thus selected 400 and 800 µM as the low and high concentrations, respectively, in our study. As shown in Fig. 1B, when hUC-SMCs at passage 2 or 3 were incubated with 400 and 800 µM indoxyl sulfate for 4 days, the percentage of cells expressing positive surface marker APC-CD73, FITC-CD90, and PerCP-cy5.5-CD105 in hUC-MSCs decreased significantly compared to the control group. This indicates that indoxyl sulfate has a negative effect on the mesenchymal stem cell marker expression.
sulfate up to 4 days, the percentage of cells expressing positive surface marker CD73, CD90, and CD105 were not significantly changed when compared with the control. Also, there was no significant difference in the percentage of cells expressing negative surface marker (data not showed).

**Effects of indoxyl sulfate on cell viability**

The toxicity of indoxyl sulfate to hUC-SMCs was assessed by Trypan blue exclusion and MTT assay. As shown in Fig. 2A, the cell viability was over 93% in the control group, and there was no significant difference in the presence of up to 800 µM indoxyl sulfate at day 2 or 4, as compared to control. MTT assay was also performed to evaluate in vitro cytotoxicity of indoxyl sulfate, and confirmed that hUC-MSCs viability was also not significantly compromised by indoxyl sulfate at 400 and 800 µM for 12 and 24 h (Fig. 2B). The above data demonstrated that there was no significant cytotoxicity when UC-MSCs were exposed to indoxyl sulfate up to 800 µM within 4 days.

**Effects of indoxyl sulfate on hUC-MSCs apoptosis**

To further confirm the cytotoxicity of the concentration of indoxyl sulfate that we applied in present studies, we next examined the effects of indoxyl sulfate on the cell apoptosis by Annexin V-FITC/PI staining kit in hUC-MSCs. We found that there were no any significant differences in the apoptotic cells between indoxyl sulfate and their control neither at the short time nor at the longer time. As shown in Fig. 3, treatment with 400 and 800 µM indoxyl sulfate for 12 and 24 h did not result in significant increase in the percentage of early-stage apoptotic cells, Annexin V-FITC⁺/PI⁻, compared to their control, respectively. No significant
differences in terms of the percentage of apoptotic cells were detected between the control and the indoxyl sulfate treated groups up to 2, 4 and 8 days (data not shown). The results thus corroborating the observations that indoxyl sulfate does not compromise hUC-MSCs viability.

Effects of indoxyl sulfate on hUC-MSCs growth and proliferation

Prompted by the report that indoxyl sulfate can inhibit proliferation of human bone marrow-derived mesenchymal stem cells [17], we examined its effects on the growth and proliferation of hUC-MSCs. As shown in Fig. 4A, exposure of hUC-MSCs with indoxyl sulfate do not show prominent alteration in the morphology of UC-SMCs, but decrease in cell growth and cell density were seen when cells were exposed to 400 and 800 µM indoxyl sulfate for 4 days. The suppression of cell proliferation was confirmed by cell number (Fig.4B) and Ki-67 nuclear immunostaining (Fig.4C).

Effects of indoxyl sulfate on hUC-MSCs senescence

To evaluate the effect of indoxyl sulfate on cellular senescence of hUC-MSCs, senescence-associated β-galactosidase (SA-β-gal) activity was tested, the senescent cells are reflected by with high β-galactosidase activity. Because it’s inhibitory effects on cell proliferation and the
quite lower cell density were seen when cell treated with 800µM indoxyl sulfate for 8 days, this might affect the accuracy of cell senescence assay, so the concentration at 400µM was used in this study. The images in Fig. 5 showed a significant increase in the percentage of cells with high SA-β-gal activity in cells treated with 400 µM indoxyl sulfate compared with
their control at 2, 4, and 8 days, respectively. Our results provide the first definite evidence that indoxyl sulfate could promote cellular senescence in hUC-MSCs in vitro.

Effects of Indoxyl sulfate on the hUC-MSCs-induced generation of CD4+CD25+FoxP3+ regulatory cells

In patients with ESRD, inflammation is strongly associated with an increased risk of atherosclerotic disease. Uremia-associated changes in the immune system have been linked to the major causes of death in patients with the ESRD-cardiovascular disease and infection [26]. CD4+CD25+FoxP3+ regulatory T cells (Tregs) are potent suppressor of inflammation. Bone marrow-derived MSCs are able to induce Tregs from peripheral blood mononuclear cells [27, 28]. Recently, hUC-MSCs exhibited suppressive effects on peripheral immune inflammation [29]. These data suggest hUC-MSCs can induce Tregs, and indoxyl sulfate can inhibit this process. To test these ideas, peripheral blood mononuclear cells (PBMCS) from the healthy volunteers were cultured with hUC-MSCs in the presence or absence of indoxyl sulfate and the results were analyzed by flow cytometry. As shown in Fig. 6A, there was a
A significant increase in the generation of Tregs after 4 days of culture, the effect compromised by pretreatment of hUC-MSCs with 400 µM indoxyl sulfate for 2 days. A significant decrease in the percentage of the generation of Tregs in PBMCs from the healthy volunteers was observed. hUC-MSCs were pretreated with 400, 800 µM or without indoxyl sulfate for 2 days, and then PBMCs from healthy volunteers were co-cultured with pretreated hUC-MSCs for 4 days. PBMCs without co-culture were used as a control. (A). Representative images for flow cytometry dot plots and histogram of PBMCs. Upper panel: dot plots illustrating CD4+CD25+ FoxP3+ regulatory T cells by flow cytometry. Lower Panel: dot plots illustrating CD4+CD25+FoxP3+ Tregs (B). Summarized data of the percentage of CD4+CD25+FoxP3+ regulatory T cells normalized to control. n = 8 batches of cells, **P < 0.001 vs. control. NS no significance, *P<0.05 vs. without pretreatment by indoxyl sulfate.
demonstrated when hUC-SMCs was pretreated with 800 µM indoxyl sulfate (Fig. 6B). Our results demonstrated, for the first time, that indoxyl sulfate can inhibit the ability of hUC-SMCs to promote Tregs development from PBMCs in vitro.

Discussion

Recently, protein-bound uremic toxins, such as indoxyl sulfate and p-cresyl sulfate, which are poorly removed by current dialysis techniques, have been associated with cardiovascular mortality in CKD and/or dialysis patients [6]. It was reported that serum concentrations of p-cresyl sulphate and indoxyl sulfate were increased to the range of 100 to 500 µM and 100 to 1000 µM in patients with CKD stage 4 to 5, respectively [30]. Several in vitro studies have demonstrated that protein-bound uremic toxins, especially indoxyl sulfate, induce vascular inflammation, endothelial dysfunction, and vascular calcification, which may explain the relatively poor prognosis of CKD and dialysis patients [23, 31, 32]. Over the past decade, advances in the field of regenerative medicine allowed for the development of cell therapies suitable for kidney repair. During stem cell treatment, there are various factors that affect the therapy efficiency. Uremic toxins are known to affect the regenerative properties of tissue-resident and circulating stem cells, thus appearing to be a limiting factor for stem cell-based approaches in treating CKD. Indoxyl sulfate is one of most important protein-bounded uremic toxins in CDK patients. Earlier studies have showed that indoxyl sulfate can significantly inhibit cell proliferation [17], alter procalcific phenotype [33], and causes other pathological changes, such as decreased osteogenic potential in human bone marrow-derived mesenchymal stem cells [34]. However, the effects of indoxyl sulfate on the human umbilical cord-derived mesenchymal stem cells are not well known. Our present study demonstrates that indoxyl sulfate inhibited proliferation of hUC-MSCs, induced their senescence and compromised their ability to stimulate Treg development. Whereas, incubation with a high concentration of indoxyl sulfate at 800 µM up to 4 days did not affect the percentage of cells expressing positive surface marker CD73, CD90, and CD105 in hUC-SMCs. Our present results support the observation that the isolated and expanded bone marrow-derived MSCs from ESRD patients and healthy controls are phenotypically similar [35]. It was found that CKD in model rats had a small effect on the gene expression and differentiation of MSCs [36]. However, long-term exposure to the uremic toxin may affect the surface marker and gene expression of MSCs derived from bone marrow and umbilical cord. Furthermore, our current studies also provide the evidence, for the first time, that indoxyl sulfate promotes cell senescence of hUC-MSCs in vitro. The results of our present studies also reach an agreement with the report that mesenchymal stem cells from rats with chronic kidney disease, exhibit premature senescence and loss of regenerative potential from CKD model rats in vivo [37]. In a study conducted by Idziak et al. [17] showed that treatment with indoxyl sulfate did not induce cell apoptosis but resulted in significant decrease in DNA synthesis by 34% at 200µM and by 47% at 1000µM in human bone marrow-derived MSCs in vitro, respectively. Our present study clearly showed the similarity of suppressive effects of indoxyl sulfate on cell proliferation of hUC-MSCs in vitro. Therefore, the damaging effect of uremic toxins on stem cells, such as inhibition of cell proliferation and promotion of cellular senescence, appears to be a major limitation for stem cell-based regenerative medicine approaches for treating CKD. In addition, it should be acknowledged that injected, healthy donor-derived cells are suddenly exposed to an altered milieu of various uremic conditions, resulting in cell phenotypic changes and functional impairment once injected in patients with CKD.

In addition to these regenerative properties, MSCs hold an immunoregulatory capacity and elicit immunosuppressive effects in a number of situations. In vitro, MSCs inhibit cell proliferation of T-cells, B-cells, natural killer cells (NK), and dendritic cells (DC). Moreover, MSCs can stop a variety of immune cell functions: cytokine secretion and cytotoxicity of T and NK cells; B cell maturation and antibody secretion; DC maturation and activation; as
well as antigen presentation [38]. MSCs can also affect the regulatory T cells (Tregs). It has been shown that co-culture of MSCs and peripheral blood mononuclear cells (PBMCs) leads to the generation of functional regulatory T cells [39]. Human Tregs suppress immune responses in vitro and in vivo. Reduced suppressive function and/or number of peripheral Treg cells have been previously reported in patients with CKD [40]. At present, the analysis of phenotype, cell growth, and functional and molecular alterations induced by uremic toxins was reported only for bone marrow-derived mesenchymal stromal cells [17, 32, 41, 42]. Our results demonstrated that hUC-MSCs strongly induced the generation of CD4\(^+\)CD25\(^+\)FoxP3\(^+\) regulatory T in PBMCs from healthy volunteers when both the cells were co-cultured; whereas the induced generation of Tregs were significantly repressed when hUC-MSCs were pretreated with uremic toxin indoxyl sulfate. Our data also provide evidence that hUC-MSCs are worthy tools for the treatment of different inflammatory disease such as CKD by a generation of inflammation suppressive Tregs. However, assessment of the retained protein-bound uremic toxins in the serum and clarifying their toxic mechanisms in the treatment of CKD patients is needed.

In conclusion, our present studies provided additional evidence that the cytotoxic effects of indoxyl sulfate, one of the retained and most important protein-bound uremic toxins in patients with CKD on umbilical cord-derived MSCs. Therefore, we proposed that high efficacy dialysis of protein-bound uremic toxins removal together in treatment with an agent that has strong absorptive properties of toxins may enhance MSC-mediated kidney repair in patients with CKD.

**Disclosure Statement**

None.

**References**


