Microbial-Derived Uremic Toxins: Role in the Pathogenesis of Comorbidities in Patients with Chronic Kidney Disease

Mikhail O. Pyatchenkov*, Andrey A. Vlasov, Evgeniy V. Sherbakov, Svetlana P. Salikova

Kirov Military Medical Academy, Saint Petersburg, Russian Federation

**Aim:** to analyze the significance of microbial-derived uremic toxins (MDUT) in the pathogenesis of comorbidities in patients with chronic kidney disease (CKD).

**Key findings.** Increased excretion of nitrogen metabolism products into the intestines of patients with CKD is associated with uremic dysbiosis, changes in the metabolic activity of the gut microbiota and the leaky gut syndrome, which largely cause the accumulation of MDUT in the internal environment of the body: indoxyl sulfate, p-cresyl sulfate, trimethylamine-N-oxide, etc. The results of recent studies allow to consider these metabolites as an independent risk factor for adverse outcomes in people with CKD due to the progression of renal dysfunction to the terminal stage, as well as frequent cardiovascular, neurological, bone mineral, nutritional and other complications.

**Conclusion.** MDUT are one of the key modulators of the pathogenetic relationship between the gut and kidneys. Therapeutic manipulations with intestinal microbiota can be considered a promising strategy for preventing complications associated with uremia.

**Keywords:** uremic toxins, gut microbiota, chronic kidney disease, comorbidities

**Conflict of interest:** the authors declare no conflict of interest.


Уремические токсины микробного происхождения: роль в патогенезе коморбидной патологии у пациентов с хронической болезнью почек

М.О. Пятченков*, А.А. Власов, Е.В. Щербаков, С.П. Саликова

ФГБОУ ВО «Военно-медицинская академия им. С.М. Кирова» Министерства обороны Российской Федерации, Санкт-Петербург, Российская Федерация

Цель публикации. Проанализировать значение уремических токсинов микробного происхождения (УТМП) в патогенезе коморбидной патологии у пациентов с хронической болезнью почек (ХБП).

Основные положения. Повышенная экскреция продуктов азотистого обмена в кишечник при ХБП ассоциирована с уремическим дисбиозом, изменениями метаболической активности микрофлоры и синдромом повышенной эпителиальной проницаемости кишечника, которые во многом обуславливают накопление во внутренних средах организма УТМП: индоксил сульфата, p-крезил сульфата, триметиламин-N-оксида и др. Результаты исследований последних лет позволяют рассматривать эти соединения в качестве самостоятельного фактора риска неблагоприятных исходов у лиц с ХБП вследствие прогрессирования дисфункции почек до терминальной стадии, а также частых сердечно-сосудистых, неврологических, минерально-костных, алиментарных и других осложнений.

Выводы. УТМП являются одними из ключевых модуляторов перекрестной патогенетической взаимосвязи между кишечником и почками. Воз действие на кишечную микрофлору можно считать перспективной стратегией предупреждения осложнений, связанных с уремией.

Ключевые слова: уремические токсины, микрофлора, хроническая болезнь почек, коморбидная патология

Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

**Introduction**

As the glomerular filtration rate decreases, the ability of the kidneys to remove metabolic products gradually decreases, which leads to the accumulation in the body of various substances called uremic toxins (UTs) [1]. Depending on the place of origin, UTs are classified into endogenous, exogenous or microbial [2].

Metabolomic studies have allowed to establish that in patients with CKD, a significant part of UTs is produced precisely with the participation of the intestinal microbiota [3, 4]. Indoxyl sulfate (IS), p-cresol sulfate (PCS), trimethylamine-N-oxide (TMAO), indole-3-acetic acid (IAA), p-cresyl glucuronide and phenylacetylglutamine are the most studied among all the currently identified MDUT [5]. It has been shown that MDUT exhibit biological activity and, therefore, can have a pathogenic effect on various types of cells. In individuals with CKD, the levels of MDUT and their precursors increase in proportion to the decline of kidney function, and are also closely associated with the risk of adverse outcomes due to frequent cardiovascular, neurological, bone mineral, nutritional and other complications [1, 6]. These organ-specific effects are mediated by various molecular mechanisms and signaling pathways, such as aryl hydrocarbon receptor (AhR)/nuclear factor-κB (NF-κB); mitogen activated protein kinase (MAPK) signaling; peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α); heme oxygenase-1 (HO-1); nuclear factor erythroid 2-related factor 2 (Nrf2); runt-related transcription factor 2 (RUNX2); bone morphogenic protein 2 (BMP2); transcription factor Sp7 (Osterix); notch signaling; autophagy effectors; microRNAs; and reactive oxygen species induction [7].

This review briefly presents the current results of clinical and experimental studies demonstrating the possible pathogenetic role of MDUT in the progression of renal failure, as well as the development of comorbidities in patients with CKD.

**Uremic dysbiosis**

Given the key role of the kidneys in maintaining the body’s homeostasis, it is natural that a violation of their function inevitably affects the functioning of other organs and systems, including the intestinal microbiota [8]. The results of numerous studies indicate that in patients with various nephropathies, the intestinal microbiota undergoes a transformation from a symbiotic state to a dysbiosis one, which is accompanied by changes in its metabolic activity [9].

It was found that in CKD, compared with healthy individuals, there is an increase in the number of Proteobacteria at the type level, while Actinobacteria and Firmicutes decrease. At the family level, there is an increase in the number of Enterobacteria and Corynebacteria, at the genus level — Enterococci and Clostridium. As CKD progresses, there is a tendency to increase the detected changes in the intestinal microbiota, as well as their differences depending on the etiology of renal failure and the variant of renal replacement therapy (hemodialysis, peritoneal dialysis or kidney transplantation) [10].

A decrease in the excretion of nitrogen metabolism products with urine, the main one among which is urea, leads to an increase in their entry into the gastrointestinal tract, which causes adaptive colonization of bacterial families expressing urease, uricase, tryptophanase and other enzymes involved in the synthesis of UTs [11]. Under the influence of microbial urease, urea undergoes hydrolysis with the formation of a large amount of ammonium hydroxide, resulting in an increase in the pH of the intraluminal contents of the intestine, irritation and local leukocyte infiltration of its mucous membrane, as well as hyperproduction of cytokines with subsequent violation of the structure and integrity of the skeleton and transmembrane proteins of tight junctions between neighboring enterocytes of the intestinal epithelium [12]. The effects of uremia are aggravated against the background of strict dietary restrictions of CKD patients, drug-induced polypharmacy, sedentary lifestyle, fluid intake restrictions and disorders of intestinal motility.

Enhanced generation of MDUT is also contributed by the proliferation of proteolytic bacterial species and entry in the colon of aromatic amino acids not absorbed into the small intestine [13]. It has been established that it is as a result of bacterial catabolism of tryptophan, phenylalanine, tyrosine and quaternary amines (betaine, L-carnitine and phosphatidylcholine) that the precursors of IS, PCS and TMAO are formed [14]. In addition, patients with CKD are characterized by a decrease in the number of bacteria synthesizing short-chain fatty acids, which have a wide range of immunoregulatory and metabolic functions [15].

The result of the above changes is the development of a leaky gut syndrome with uncontrolled transport into the bloodstream of a number of immunogenic substances generated by an aberrant microbiota, as well as activation of chronic systemic inflammation and oxidative stress, which are universal mechanisms for the development of many diseases [16, 17].
System effects of microbial-derived uremic toxins

Currently, there is no doubt that gut dysbiosis is an important factor in the pathogenesis of common socially significant diseases, such as obesity, diabetes mellitus, cardiovascular pathology and CKD [5, 18–20]. MDUT is considered as one of the major modulators of the connection between gut dysbiosis and these diseases occurrence and progression. The susceptibility of various tissues to UTs is determined by their type, interstitial concentration, as well as tissue perfusion and cellular permeability [21]. Unlike bacteria and their endotoxins, which induce inflammation and the synthesis of reactive oxygen species (ROS), the adverse effects of MDUT are caused, including, by their direct effect on cells [5, 7]. It has been established that some UTs, in particular, the advanced glycation end products (AGEs), have an effect on survival, migration and differentiation of endotheliocytes by linking with specific surface receptors (RAGE) [5]. In the study of H. Watanabe et al., it was shown that uptake by the renal tubules' epithelial cells some UTs (IS and PCS) can be mediated by organic anion transporters (OAT1 and OAT3) [22]. The ability of IS to affect a wide range of targets largely depends on the possibility of its transport through the plasma membrane and cytoplasmic contacts with AhR [21]. In addition, in patients with end stage renal disease, the low-molecular-weight water-soluble TMAO molecule is effectively removed by dialysis, in contrast to the protein-bound IS and PCS [23]. Thus, intracellular accumulation of MDUT or changes in their toxicokinetic in patients with CKD may be associated with varying degrees of negative effects described below.

Microbial-derived uremic toxins and chronic kidney disease progression

A growing number of publications indicate the important role of MDUT in the progression of renal failure. It was found that the accumulation of IS in the cells of the renal tubules disrupts their antioxidant system, and also enhances the renal expression of the genes involved in tubulointerstitial fibrosis, such as tissue metalloproteinase inhibitor, transforming growth factor β1 (TGF) and type I alpha-1 collagen [8, 24]. O. Ichii et al. in vitro examined toxic effect of IS on the cellular elements of the kidneys and found pathological changes of podocytes were, including wrinkling of the glomerular basement membrane, podocytes foot effacement and the formation of cytoplasmic vacuoles [25]. A significant effect on the structural and functional state of renal tissue has also been proven for other MDUT. A number of studies indicate that PCS and TMAO in experimental animals with CKD contribute to increased tubulointerstitial fibrosis and renal dysfunction [26, 27]. IAA, synthesized by intestinal bacteria from tryptophan, has similar effects by inducing the proinflammatory enzyme cyclooxygenase-2 and oxidative stress [28]. Epigenetic modification of some specific genes, apparently, may be another important nephrotoxic mechanism of MDUT. It has been shown that IS and PCS are involved in renal tissue remodeling, reducing the expression of nephroprotective factor klotho in the renal tubules [29, 30]. Elevated levels of MDUT in patients with CKD predict further progression of renal failure, cardiovascular events and all-causes mortality [31–33].

Cardiovascular diseases

Cardiovascular pathology is one of the leading causes of increased morbidity and mortality in patients with CKD. This is explained by high prevalence of both traditional and atypical cardiovascular risk factors, among which intestinal dysbiosis, MDUT and inflammation are considered the most significant [34]. The products of abnormal microbial metabolism in high concentrations have a direct toxic effect on cardiomyocytes, smooth muscle and vascular endothelial cells, participating in the remodeling of the myocardium and blood vessels, in the pathogenesis of atherosclerosis, hypertension and heart failure. Data obtained in in vitro experiments indicate that IS stimulates collagen synthesis by cardiac fibroblasts and cardiomyocyte hypertrophy. It is assumed that these effects are mediated by activation of mitogen activated protein kinase (MAPK) and the NF-kB signaling pathway [35]. It was found that PCS also causes significant structural and functional changes in the myocardium. PCS in CKD rats model led to an increase in the coefficient of cardiomyocyte apoptosis due to increased activity of caspase-3, as well as the production of reactive oxygen species and NADPH oxidase [36].

Intestinal dysbiosis contributes to the development of the most important CKD risk factor — arterial hypertension. N. Oshima et al. have shown that various types of UTs, including IS, activate bulbo-spinal neurons in the rostral ventrolateral medulla, a key area regulating blood pressure [37]. A number of studies have demonstrated that in patients with CKD, a high level of IS was associated with left ventricular myocardial hypertrophy, QT interval prolongation, increased risk of heart failure, and also serves as a strong predictor of overall and cardiovascular mortality [38].

Currently, there is strong evidence of the role of MDUT in accelerating the progression
of endothelial dysfunction, atherosclerosis and vascular calcification as a result of induction of systemic inflammation and oxidative stress [39]. In patients with various stages of CKD, a relationship was found between the content of some MDUT (IS, PCS) and carotid artery intima-media thickness, carotid-femoral pulse wave velocity and degree of aortic calcification [40]. IS can inhibit endothelial progenitor cells mediated neovascularization of ischemic tissues as well as enhance platelet aggregation and thrombus formation [41]. In patients with CKD higher blood levels of PCS significantly correlate with the severity of coronary arteriosclerosis, peripheral artery pathology and dysfunction of vascular access for hemodialysis [42, 43]. In this regard, the most convincing data are currently available for TMAO, the increased concentration of which, according to the results of large systematic review, was associated with a higher risk of major adverse cardiovascular events, as well as general and cardiovascular mortality [33]. Thus, individual and combined pathophysiological mechanisms involving MDUT may underlie the development and progression of cardiovascular pathology in individuals with CKD.

**Nutritional status. Sarcopenia**

The intestinal microbiota is necessary for the normal metabolism of nutrients and maintaining the energy balance of the body. Therefore, gut dysbiosis can make a significant contribution to the development of nutritional disorders in patients with CKD. Intestinal microflora affects the nutritional status of the host organism through microbial metabolites, systemic inflammation, appetite regulation, acidosis and various hormonal disorders. J. Hu et al. determined that anthropometric indicators, including handgrip strength, mid-upper arm circumference, mid-upper arm muscle circumference, and body mass index, in patients on dialysis negatively correlated with the level of conditionally pathogenic bacteria (*Escherichia* spp.) involved in the synthesis of UTs [44]. In turn, L. Caldiroli et al. found a positive association of serum PCS level with protein energy wasting syndrome in elderly patients with advanced CKD [45].

Currently, significant evidence has been obtained of that the MDUT is involved in the pathogenesis of uremic sarcopenia. The results of experimental studies show influence of MDUT on the structural and functional state of skeletal muscle tissue. E. Sato et al. visualized significant accumulation of IS and PCS in skeletal muscle of mice with adenine-induced CKD and established linear correlation between their levels and the severity of muscular atrophy [46]. IS has a direct toxic effect on myoblasts, reducing their viability and increasing cell apoptosis [47]. IS also induced mitochondrial dysfunction by decreasing the expression of PGC-1 and inducing autophagy in addition to decreasing mitochondrial membrane potential [48]. In addition, MDUT reduced the functional activity of myoblasts by premature termination of their differentiation, decrease myotubes formation or occurrence of their structural anomalies [49].

The results of clinical studies on this topic are controversial and differ depending on the type of studying UT [50, 51]. It should be noted that there are no convincing data for close relationship between IS and PCS levels and the severity of skeletal muscle loss in patients with CKD [46, 52].

**Mineral bone disorders**

Increasing evidence indicates that high levels of MDUT may play an important role in the pathogenesis of mineral bone disorders in patients with CKD, disrupting processes of the osteoblastogenesis and osteoclastogenesis, inhibiting bone mineralization, alkaline phosphatase activity, type I collagen transcription and the expression of other genes associated with bone formation [38, 53]. It is known that the uremic environment deteriorates the bone response to the parathyroid hormone (PTH) [54]. J. Hirata et al. found that dietary supplements with indole increase the level of IS in the blood and lead to further bone remodeling in rats after parathyroidectomy [55]. Concentration of IS in hemodialysis patients negatively correlates with markers of bone formation regardless of intact PTH level [56]. These data suggest that MDUT may worsen low bone metabolism by inhibiting bone formation through mechanisms unrelated to skeletal resistance to PTH. In pre-dialysis CKD patients, a relationship was found between serum concentration of IS, fibroblast growth factor-23 (FGF23) and the bone fibrosis [57, 58]. Another study showed that in 154 patients at CKD stages 2-5D, serum sclerostin (a predictor of increased fracture risk) independently correlates with IS, PCS and β2-microglobulin levels [59]. Thus, MDUT in patients with CKD may modify bone metabolism. However, the exact mechanisms of this influence remain unknown and require further study.

**Anemia**

MDUT blood accumulation leads to disruption of the synthesis of erythropoietin (EPO) by kidney peritubular fibroblasts due to suppression of EPO gene transcription [60]. In addition, IS inhibits the activation of hypoxia-inducible factor (HIF), which is the main regulator of hypoxic EPO production [61]. Another possible mechanism
of MDUT-induced anemia described in the study by Y. Adelibieke et al., is the suppression of intracellular pathways of EPO receptor activation, which may contribute to their resistance to EPO [62]. It has been established that IS in vitro stimulates eryptosis (programmed death of erythrocytes) [63], and also disrupts iron metabolism by regulating hepcidin synthesis [64]. Meanwhile, observational studies have not shown any association between MDUT levels and anemia in patients receiving dialysis treatment [65]. Future research in this field should determine the exact role of MDUT in the development of anemia in CKD.

**Cognitive dysfunction**

Despite the buffering function of the blood-brain barrier, decrease MDUT clearance by damaged kidneys leads to their gradual accumulation in brain tissues [46]. In patients with CKD MDUT have a direct neurotoxic effect with the progression of cognitive disorders [7]. IS is also found in the cerebrospinal fluid [66]. In vitro studies have shown that both low-molecular and protein-bound UTs reduce the viability of neuronal cells via inducing inflammation, oxidative stress and apoptosis [67, 68]. In mice with experimentally reproduced CKD, increase level of IS in the brainstem is associated with decrease of neurotransmitters content (norepinephrine, serotonin and dopamine), which was accompanied by various neurobehavioral disorders, including apathetic behavior, increased sensitivity to stress, decreased motor and exploratory activity and impaired spatial memory and coordination of movements [69]. In addition to direct neurotoxic effects MDUT play an important role in the pathogenesis of cerebrovascular diseases due to its effect on vascular tone and blood pressure [34]. Moreover, endothelial dysfunction and hemostatic disorders induced by UTs can cause cerebral microvascular dysfunction, which is a frequent cause of a significant proportion of cases of dementia and stroke [39]. In clinical studies, it has been proven that higher serum levels of IS and IAA in CKD individuals increase the risk of various cognitive disorders and dementia [7, 39, 70].

**Other organ damage**

Currently, the possible involvement of MDUT in the development of various metabolic disorders that often accompany CKD is being discussed. L. Koppe et al. showed that PCS may induce insulin resistance in cultured muscle and fat cells. Intraperitoneal injections of PCS for a 4 weeks similarly induced insulin resistance with ectopic lipid redistribution in skeletal muscle and liver in mice with normal renal function [71]. In rats with subtotal nephrectomy, the accumulation of IS in adipose tissue is accompanied by oxidative stress, an increase in the content of lipid peroxidation products and activate insulin signaling [72]. The hepatotoxic effect of MDUT is manifested in their ability to activate in vitro apoptosis of hepatocytes [73]. MDUT are probably associated with the development of uremic pruritus in patients with end-stage CKD, since an increase the dialysis dose, as well as the use high-flux dialyzers and biocompatible membranes often results to an improvement in symptoms [74]. It is assumed that IS may also be involved in lung tissue damage in renal failure [75].

The results of numerous studies indicate that dietary changes, the use of drugs that normalize the intestinal microflora (prebiotics, probiotics, synbiotics), sorbents (AST-120) and some other therapeutic interventions can reduce the level of MDUT and the activity of chronic systemic inflammation in patients with CKD [6, 8, 12, 17]. Although some of these approaches seem promising and indirectly confirm the pathogenic role of MDUT, currently none of them has shown a significant effect on cardiovascular outcomes or mortality in randomized controlled trials and therefore cannot be recommended for clinical use.

**Conclusion**

Advances in modern science have made it possible to establish that MDUT are one of the key modulators of the cross pathogenetic relationship between the intestine and kidneys, and effort to reduce their concentration seems to be a reasonable strategy for preventing complications associated with uremia. Thus, exposure to the intestinal microbiota can become a widely available non-invasive therapeutic approach with the potential to reach a large number of patients. However, further large-scale clinical trials are needed to confirm the safety and efficacy of these approaches in improving outcomes and survival in patients with CKD.
References / Reviews


30. Don L., Salle M., Cerini C., Poiettini S., Goudouin B., Jouard-Chiche N., et al. The cardiovascular effect of the
Uric acid, indoxyl sulfate, a uraemic toxin.

A systematic review of prospective cohort studies.


Hamza E., Metzinger L., Metzinger-Le Meuth V. Uremic toxins affect erythropoiesis during the course of...


Information about the authors

Mikhail O. Pyatchenkov* — Cand. Sci. (Med.), Senior Lecturer of the Department of Nephrology and Blood Purification, Kirov Military Medical Academy. Contact information: pyatchenkovMD@yandex.ru; 194044, Saint-Petersburg, Academician Lebedev str., 6. ORCID: https://orcid.org/0000-0002-5893-3191

Andrey A. Vlasov — Cand. Sci. (Med.), Resident of 2nd Therapy Department of Postgraduate Education, Kirov Military Medical Academy. Contact information: vlasovandrej@mail.ru; 194044, Saint-Petersburg, Academician Lebedev str., 6. ORCID: https://orcid.org/0000-0002-7915-3792

Evgeniy V. Sherbakov — Nephrologist of the Department of Nephrology and Blood Purification, Kirov Military Medical Academy. Contact information: evgenymedagmail.ru; 194044, Saint-Petersburg, Academician Lebedev str., 6. ORCID: https://orcid.org/0000-0002-3045-1721

* Corresponding author / Автор, ответственный за переписку

Сведения об авторах

Пятченков Михаил Олегович* — кандидат медицинских наук, старший преподаватель кафедры нефрологии и эффе рентной терапии ФГБВОУ ВО «Военно-медицинская академия им. С.М. Кирова» Министерства обороны Российской Федерации. Контактная информация: pyatchenkovMD@yandex.ru; 194044, г. Санкт-Петербург, ул. Академика Лебедева, 6. ORCID: https://orcid.org/0000-0002-5893-3191

Власов Андрей Александрович — кандидат медицинских наук, соискатель 2-й кафедры (терапии усовершенство вания врачей) ФГБВОУ ВО «Военно-медицинская акаде мия им. С.М. Кирова» Министерства обороны Российской Федерации. Контактная информация: vlasovandrej@mail.ru; 194044, г. Санкт-Петербург, ул. Академика Лебедева, 6. ORCID: https://orcid.org/0000-0002-7915-3792

Щербаков Евгений Вячеславович — врач нефролог клиники нефрологии и эффе рентной терапии ФГБВОУ ВО «Военно-медицинская академия им. С.М. Кирова» Министерства обороны Российской Федерации. Контактная информация: evgenymedagmail.ru; 194044, г. Санкт-Петербург, ул. Академика Лебедева, 6. ORCID: https://orcid.org/0000-0002-3045-1721
Svetlana P. Salikova — Dr. Sci. (Med.), Associate Professor of the 2nd Therapy Department of Postgraduate Education, Kirov Military Medical Academy.
Contact information: salikova.1966@bk.ru;
194044, Saint-Petersburg, Academician Lebedev str., 6.
ORCID: https://orcid.org/0000-0003-4839-9578

Submitted: 10.01.2023 Accepted: 01.03.2023 Published: 30.06.2023
Поступила: 10.01.2023 Принята: 01.03.2023 Опубликована: 30.06.2023