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EFEKTI KADMIJUMA NA TRANSPORTNE PROCESE U PROKSIMALNIM TUBULSKIM ĆELIJAMA BUBREGA

Teodora Pejović[1], Sanjin Kovačević[1], Jelena Nešović Ostojić[1*]

[1] Institut za patološku fiziologiju „dr Ljubodrag Buba Mihailović", Medicinski fakultet Univerziteta u Beogradu, Beograd, Republika Srbija

[*] Korespondencija: jelena.nesovic-ostojic@med.bg.ac.rs

SAŽETAK

Kadmijum (Cd) je ekstremno toksičan metal koji je u prirodi široko rasprostranjen. Zbog svojih povoljnih osobina masovno se upotrebljavao u industriji za izradu alkalnih baterija, akumulatora, pigmenata, bojenih legura. Ipak, pokazano je da izlaganje kadmijumu u malim koncentracijama dovodi do oštećenja brojnih organa i organskih sistema te se upotreba ovog metala u industriji smanjuje, a zamenjuju ga drugi, manje štetni materijali. Danas je važan izvor izloženosti kadmijumu sagorevanje fosilnih goriva i konzumiranje cigareta. Brojne studije ispitivale su štetne efekte kadmijuma i one ističu bubrege, jetru i gonade kao organe koji trpe najveća oštećenja. Bubrezi, kao glavno mesto deponovanja kadmijuma u organizmu, u najvećoj meri izloženi su njegovim toksičnim efektima. U proksimalnim tubulskim ćelijama bubrega izlaganje kadmijumu remeti transportne procese. Iako se smatra da je jonizovani kadmijum (Cd^{2+}) u najvećoj meri odgovoran za oštećenja koja nastaju, ne može se zanemariti ni uloga kompleksa kadmijuma i metalotioneina (Cd-MT). Peritubularno izlaganje jonizovanom kadmijumu dovodi indirektno do smanjenja aktivnosti Na^+/L-alanin kotransportera i smanjenja brzine spore repolarizacije luminalne membrane, dok kompleks Cd-MT dovodi i do direktne i do indirektne inhibicije ovog transportera. Takođe, Cd-MT kompleks inhibira aktivnost Na^+/glukoza kotransportera. Izlaganje kadmijumu dovodi i do smanjenja preuzimanja niskomolekularnih proteina putem olakšane endocitoze što je praćeno mikroalbuminurijom.

Ključne reči: kadmijum, proksimalni tubuli, transport, nefrotoksičnost.

Uvod

Kadmijum (Cd) je teški metal koga su otkrili 1817. godine, nemački hemičari *Friedrich Strohmeyer* i *Karl Hermann* kao nečistoću u cink-karbonatu. U prirodi ovaj element se može naći u sastavu Zemljine kore, emituje se pri vulkanskim erupcijama, šumskim požarima, eroziji stena u reke i mora (1,2). Značajna količina kadmijuma nalazi se u fosilnim gorivima pa se njihovim sagorevanjem emituje u atmosferu. U prošlosti kadmijum je imao različite namene – u obliku kadmijum jodida koristio se za lečenje otoka zglobova, promrzlina, a zbog svoje povoljne osobine da teško korodira koristio se za premazivanje legure čelika i gvozđa. Široko se primenjivao u industriji: za izradu alkalnih baterija, akumulatora, pigmenata, bojenih legura, plastike (1-3). Danas se prema podacima Američke agencije za registrovanje toksičnih supstanci i bolesti (engl. A*gency for Toxic Substances and Disease Registry* - ATSDR) kadmijum nalazi na sedmom mestu prioritetne liste opasnih supstanci zbog čega se njegova upotreba smanjuje. Ova agencija kao glavni izvor izloženosti kadmijumu ističe konzumiranje cigareta kod pušača, dok se nepušači najčešće izlažu konzumacijom hrane zagađene kadmijumom (4). Voda u blizini industrijskih objekata koji koriste kadmijum sadrži povećane koncentracije ovog metala, a izloženost putem vazduha je takođe značajna samo u ovim područjima. Do profesionalne izloženost može doći u rudnicima ili tokom proizvodnje i prerade sirovina koje sadrže kadmijum (4).

Brojne studije pokazale su da kadmijum uzrokuje oštećenja različitih organa, a svoju toksičnost ispoljava i pri izlaganju niskim koncentracijama

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THE EFFECTS OF CADMIUM ON THE TRANSPORT PROCESSES IN PROXIMAL TUBULAR CELLS OF KIDNEYS

Teodora Pejovic[1], Sanjin Kovacevic[1], Jelena Nesovic Ostojic[1*]

[1] Institute of Pathophysiology "Dr Ljubodrag Buba Mihailovic", Faculty of Medicine, University of Belgrade, Belgrade, Republic of Serbia

[*] Correspondence: jelena.nesovic-ostojic@med.bg.ac.rs

SUMMARY

Cadmium (Cd) is an extremely toxic metal that is widespread in nature. Due to its favorable properties, it was widely used in the industry for the production of alkaline batteries, accumulators, pigments, and colored alloys. However, it has been shown that exposure to low concentrations of cadmium leads to damage to many organs and organ systems, and the use of this metal in industry is reduced, and it is replaced by other, less harmful materials. Today, fossil fuel combustion and cigarette consumption are important sources of cadmium exposure. Numerous studies have examined the toxic effects of cadmium and they highlight the kidneys, liver, gonads as the organs that suffer the most damage. The kidneys, as the main place of cadmium storage in the body, are mostly exposed to its toxic effects. In the proximal tubular cells of the kidney, exposure to cadmium disrupts transport processes. Although ionized cadmium (Cd^{2+}) is thought to be largely responsible for the damage that occurs, the role of the cadmium and metallothionein complex (Cd-MT) cannot be ignored. Peritubular exposure to ionized cadmium indirectly leads to a decrease in the activity of the Na^+/L-alanine cotransporter and a decrease in the rate of slow repolarization of the luminal membrane, while the Cd-MT complex leads to both direct and indirect inhibition of this transporter. Also, the Cd-MT complex inhibits Na^+/Glucosa cotransporter activity. Exposure to cadmium also leads to a decrease in the endocytic uptake of low molecular weight proteins, which is accompanied by microalbuminuria.

Key words: cadmium, proximal tubules, transport, nephrotoxicity

Introduction

Cadmium (Cd) is a heavy metal that was discovered in 1817 by German chemists *Friedrich Strohmeyer* and *Karl Hermann* as an impurity in zinc-carbonate. In nature, this element can be found in the Earth's crust, and it is released during volcanic eruptions, forest fires, coastal erosion (1,2). Significant quantities of cadmium are present in fossil fuels, and therefore, emissions of cadmium are caused by their combustion. In the past, cadmium had different uses – it was used as cadmium iodide for the treatment of swollen joints, frostbite, and due to its favorable properties regarding corrosion, cadmium coatings were used to protect alloys of steel and iron against corrosion. It was widely applied in industry: for the production of alkaline batteries, accumulators, pigments, colored alloys, plastics (1-3). Today, according to the US Agency for Toxic Substances and Disease Registry (ATSDR), cadmium ranks seven on the priority list of hazardous substances, and therefore, its usage has been reduced. This agency stresses cigarette consumption as the main source of cadmium exposure in smokers, while non-smokers are most frequently exposed to cadmium via food that is contaminated by cadmium (4). Water that is near industrial facilities that use cadmium contains higher concentrations of this metal, while exposure via air is also significant in these regions. Occupational exposure may happen in mines or during the production and processing of raw materials that contain cadmium (4).

(5). Mehanizmi kojima ispoljava štetno dejstvo su višestruki – vezujući se za sulfhidrilne grupe antioksidativnih enzima inhibira njihovu aktivnost, nagomilavanjem u ćeliji može izazvati disfunkciju mitohondrijalnog transportnog lanca elektrona i stvaranje reaktivnih kiseoničnih radikala što posledično dovodi do oksidativnog stresa, smanjuje koncentraciju selena u organizmu potrebnog za formiranje glutation peroksidaze, indukuje inflamaciju, apoptozu ćelija (5-7). U najvećoj meri kadmijum oštečuje bubrege, jetru i gonade ali oštećenjem su zahvaćeni i drugi organski sistemi – respiratorni, hematopoezni, kardiovaskularni, koštano zglobni (5).

Bubrezi predstavljaju glavni organ za deponovanje i toksične efekte kadmijuma. Od ukupne količine kadmijuma u organizmu, u bubrezima se nalazi 30-50%, pri čemu je glavno mesto deponovanja u ćelijama proksimalnih tubula (8). Pokazano je da u ćelijama proksimalnih tubula bubrega deponovani kadmijum remeti procese reapsorpcije različitih supstanci iz primarnog urina. Stoga, cilj ovog istraživanja je da što preciznije sumira dosadašnja znanja o uticaju kadmijuma na transportne procese u ćelijama proksimalnih tubula bubrega.

Metode

U ovom preglednom radu, radi što preciznijeg i sveobuhvatnijeg prikaza efekata kadmijuma na ćelije proksimalnih tubula, korišćena je literatura dobijena pretraživanjem MEDLINE baze podataka uz pomoć servisa PUBMED. Literatura objavljena na engleskom jeziku, u poslednjih 10 godina, dobijena je pretraživanjem sledećih ključnih reči: kadmijum, nefrotoksičnost, proksimalni tubuli.

Kadmijum – toksikokinetika i toksikodinamika

Dominantan put ulaska kadmijuma u organizam kod osoba koje nisu profesionalno izložene ovom elementu je oralni, kod profesionalno izloženih dominantan je inhalatorni put unosa, a ukupnoj ekspoziciji doprinose i apsorpcija kadmijuma preko kože i gastrointestinalnog trakta (8). Iz lumena creva kadmijum se transportuje u enterocite preko transportera za dvovalentne metale tip 1 (DMT-1, *divalent metal ion transporter 1*) i proteina za transport metala tip 1 (MTP-1, *metal transport protein 1*), a apsorpcija je moguća i vezivanjem za sulfhidrilne grupe cisteina i glutationa (9,10). Kada dospe u krv, kadmijum se transportuje eritrocitima i vezan za proteine plazme (8). *Yiling Li* i saradnici su u nedavno rađenoj studiji pokazali da je jedan od proteina ljudske plazme za koji se kadmijum vezuje u krvi apolipoprotein A-I (11). Dalje se kadmijum transportuje do svojih depoa u organizmu – bubrega, jetre i mišića (8). U akutnoj toksičnosti primarno oštećenje izazvano kadmijumom događa se na nivou jetre, dok se u hroničnoj toksičnosti dominantno oštećuju bubrezi (12). U hepatocitima kadmijum stimuliše sintezu metalotioneina (MT) sa kojima formira kompleks koji štiti ćelije od oksidativnog stresa. Smatra se da oštećenje hepatocita nastaje kada se prevaziđe kapacitet metalotioneina za puferovanje jona kadmijuma (13,14). Po odumiranju hepatocita kompleks Cd-MT se ciruklacijom transportuje do bubrega. Kadmijum je delom vezan i za druge tiolne grupe – glutation (GSH), L-cistein (L-Cis), a delom se transportuje do bubrega i kao slobodan, jonizovani kadmijum (Cd^{2+}). U bubrezima se Cd-MT, Cd-GSH, Cd-Cis i slobodan Cd^{2+} lako filtriraju kroz glomerule bubrega, a zatim se na različite načine reapsorbuju (15,16). Cd-MT na nivou proksimalnih tubula se preuzima endocitozom, Cd-Cis i Cd-GSH se preuzimaju preko apikalne i bazolateralne membrane proksimalnih tubula, a Cd^{2+} se različitim transportnim sistemima može apsorbovati (15,16). Eliminacija kadmijuma u najvećoj meri vrši se preko gastrointestinalanog trakta, a u manjoj meri se u kompleksu sa metalotioneinima eliminiše preko bubrega (6,8).

Proksimalni tubuli bubrega

Osnovna strukturna i funkcionalna jedinica bubrega je nefron koji se sastoji od glomerula i pratećih kanalica. Glomerul je izgrađen od spleta kapilara smeštenih unutar Boumanove (*Bowman's*) kapsule. Na nivou glomerula vrši se filtracija krvi i stvara se primarni urin (17). Dalje se na urinarni pol glomerula nastavlja proksimalni tubul koji ima svoj izvijugani (*pars convoluta*) i pravi deo (*pars recta*) (18). Na nivou proksimalnog tubula vrši se reapsorpcija najvećeg dela ogranskih supstanci poput aminokiselina, glukoze, albumina i niskomolekularnih proteina ali i velikog dela neorganskih supstanci: natrijuma, hlorida, fosfata, bikarbonata. Većina ovih supstanci transportuje se kotransportom sa natrijumom. Naime, na bazolateralnom polu epitelnih ćelija proksimalnog

Numerous studies have shown that cadmium causes damage of different organs and that it shows toxicity even during exposure to low concentrations (5). The mechanisms, with which it shows its harmful effects, are numerous – by binding to the sulfhydryl groups of antioxidative enzymes, it inhibits their activity, by accumulating in the cell, it can cause the dysfunction of mitochondrial electron transport chain and generation of reactive oxygen radicals resulting in oxidative stress; it reduces the concentration of selenium in the body necessary for the formation of glutathione peroxidase; it induces inflammation, cell apoptosis (5-7). It mostly damages kidneys, liver and gonads, however, other organ systems are damaged, as well – respiratory, hematopoietic, cardiovascular, musculoskeletal (5).

Kidneys present the main organ for depositing and toxic effects of cadmium. Of the total quantity of cadmium in the body, 30-50% is in the kidneys, while the main place of depositing is in proximal tubular cells (8). It has been shown that in the proximal renal tubules, deposited cadmium disturbs the processes of reabsorption of different substances from primary urine. Therefore, the aim of this study is to summarize the current knowledge about the effects of cadmium on the transport processes in proximal tubular cells of kidneys.

Methods

In this review article, we used literature that was searched in the MEDLINE database with the help of PUBMED service in order to present the effects of cadmium on proximal tubular cells in a precise and comprehensive way. The literature, which has been published in the English language during the last ten years, was obtained by searching the following key words: cadmium, nephrotoxicity, proximal tubules.

Cadmium – toxicokinetics and toxicodynamics

Oral ingestion is the predominant pathway of exposure in persons, who are not professionally exposed to this element, while inhalation is a major route of occupational exposure, and the absorption of cadmium via skin and gastrointestinal tract contribute to the total exposure (8). Cadmium is transported from the intestinal lumen into enterocytes via divalent metal ion transporter type 1 (DMT-1) and metal transport protein 1 (MTP-1), while the absorption is possible by binding to the sulfhydryl groups of cysteine and glutathione (9,10). When it enters the bloodstream, cadmium is transported via erythrocytes and it is bound to plasma proteins (8). *Yiling* and associates have shown in a recent study that one of the proteins of human plasma, which cadmium is bound to in blood, is apolipoprotein A-I (11). Furthermore, cadmium is transported to its depots in the body – kidneys, liver and muscles (8). In its acute toxicity, primary damage caused by cadmium happens in liver, while in chronic toxicity, kidneys are dominantly damaged (12). In hepatocytes, cadmium stimulates the synthesis of metallothionein (MT), with which it forms the complex that protects cells from oxidative stress. It is deemed that the damage of hepatocytes occurs when the capacity of metallothionein for buffering cadmium ions is exhausted (13,14). When hepatocytes die off, Cd-MT complex is transported to kidneys through circulation. Cadmium is partly bound to other thiol groups – glutathione (GSH), L-cysteine (L-cys), and partly it is transported to kidneys as free, ionized cadmium (Cd^{2+}). In kidneys, Cd-MT, Cd-GSH, Cd-Cys and free Cd^{2+} are easily filtered through renal glomeruli, and then they are reabsorbed in different ways (15,16). Cd-MT at the level of proximal tubules is taken up through endocytosis, Cd-Cys and Cd-GSH are taken up through apical and basolateral membrane of proximal tubules, while Cd^{2+} can be absorbed by different transport systems (15,16). Cadmium is to the greatest extent eliminated through gastrointestinal tract, while it is eliminated to the lesser extent in the complex of metallothionein via kidneys (6,8).

Proximal tubules of kidneys

Basic structural and functional unit of kidneys is a nephron which consists of glomerulus and tubules. The glomerulus is composed of a network of capillaries within the Bowman's capsule. Blood is filtered at the level of glomerulus and primary urine is produced (17). The proximal tubule begins at the urinary pole of the glomerulus and it has *pars convoluta* and *pars recta* (18). The proximal tubule is the site of reabsorption of most organic substances such as amino acids, glucose, albumin and low-molecular-weight proteins, as well as

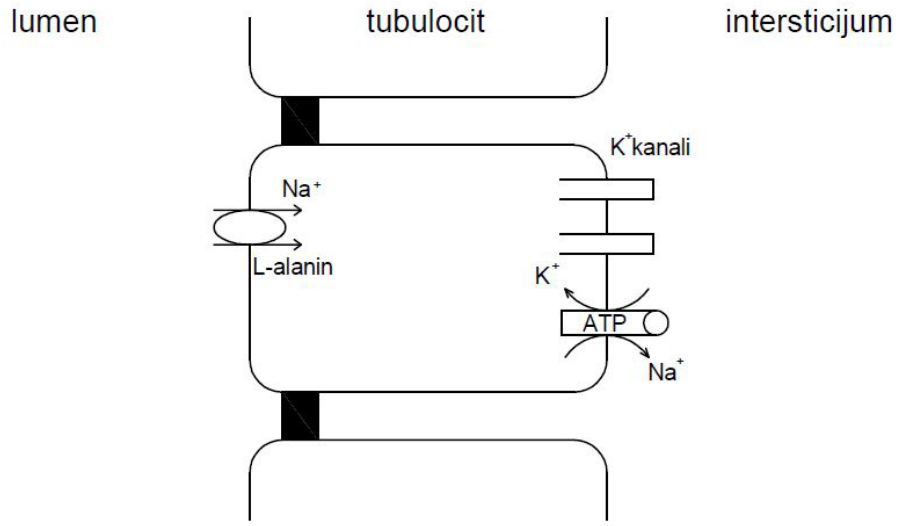
tubula postoji Na^+/K^+ ATP-azna pumpa koja izbacuje natrijum iz ćelije, a ubacuje kalijum i tako stvara hemijski gradijent za apsorpciju natrijuma na luminalnom polu ćelije. Na luminalnom polu postoje kotransporteri: Na^+/glukoza kotransporter, Na^+/amino-kiselinski kotransporter, Na^+/Cl^- kotransporter, Na^+/HCO_3^- kotransporter, preko kojih dolazi do reapsorpcije ovih supstanci, a zahvaljujući elektrohemijskom gradijentu natrijuma (19) (slika 1).

Efekti kadmijuma na proksimalne tubule bubrega

Kao što je napred spomenuto, glavno mesto deponovanja kadmijuma su ćelije proksimalnih tubula bubrega (8). Smatra se da glavnu ulogu u oštećenju ima slobodan Cd^{2+}, a da metalotioneini imaju zaštitnu ulogu i da do oštećenja dolazi kada se prevaziđe kapacitet bubrega da stvara metalotioneine. Studije rađene na miševima koji ne mogu da sintetišu metalotioneine i miševima koji mogu da ih sintetišu, pokazuju da su prvi znatno osetljiviji na toksično dejstvo kadmijuma, a kako postoje velike varijacije u ekspresiji metalotioneina kod ljudi, neki bi mogli biti predisponirani na toksična oštećenje indukovana kadmijumom (13,20). Ipak, pojedine studije sugerišu da metalotioneini u većoj meri imaju protektivno dejstvo na ćelije jetre nego na ćelije bubrega (20).

U ćelijama proksimalnih tubula bubrega vrši se reapsorpcija najvećeg dela supstanci iz primarnog urina i to uglavnom sekundarnim aktivnim transportom – kotransportom sa natrijumom (19). Stoga, nije iznenađujuće što se veliki broj studija bavio ispitivanjem uticaja kadmijuma na transportne procese u proksimalnim tubulskim ćelijama bubrega. Pošto se većina supstanci transportuje na osnovu elektrohemijskog gradijenta natrijuma, promene membranskog potencijala u velikoj meri utiču na transportne procese (21). Na bazolateralnoj membrani ćelija proksimalnih tubula nalaze se kalijumski kanali kojima se K^+ transportuje iz ćelije u intersticijum, niz gradijent koncentracije, čime se obezbeđuje hiperpolarizacija bazolateralne membrane. Bazolateralna i luminalna membrana su električno spojene, pa se ova hiperpolarizacija prenosi i na luminalnu membranu. Hiperpolarizacija luminalne membrane je važna jer obezbeđuje električni gradijent – pokretačku snagu za ulazak natrijuma u ćeliju (22,23).

Peritubulska perfuzija mikromolarnim koncentracijama kadmijuma (Cd^{2+}) dovodi do održive, reverzibilne hiperpolarizacije bazolateralne, a potom i luminalne membrane. Do hiperpolarizacije membrane najverovatnije dolazi zbog vezivanja kadmijuma za kalijumske kanale na bazolateralnoj membrani što dovodi do konformacionih promena samih kanala pa kalijum pojačano izlazi iz ćelije (24-26) (slika 2). Da hiperpolarizacija zaista nastaje kao rezultat povećane propustljivosti bazolateralnih kalijumskih kanala pokazano je njihovom blokadom nespecifičnim blokatorom barijumom, kada hiperpolarizacija izostaje (25,26). Međutim, ova hiperpolarizacija je praćena smanjenjem visine depolarizacije pokrenute aktivacijom Na^+/L^-alanin kotransportera na luminalnoj membrani (27), što bi moglo da ukaže na smanjenje reapsorpcije L-al-



Slika 1. Shematski prikaz transportnih procesa u proksimalnim tubulskim ćelijama

the great part of inorganic substances: sodium, chloride, phosphate, bicarbonate. The majority of these substances are transported by the co-transport with sodium. Namely, at the basolateral pole of epithelial cells of proximal tubule, there is Na^+/K^+ ATPase pump which extrudes sodium from the cell and imports potassium into the cell, thus creating the chemical gradient for the absorption of sodium at the luminal pole of the cell. At the luminal pole, there are co-transporters: Na^+/glucose co-transporter, Na^+/amino-acid co-transporter, Na^+/Cl- co-transporter, $Na^+/HCO3^-$ co-transporter, through which the reabsorption of these substances occurs thanks to the electrochemical gradient of sodium (19) (Figure 1).

The effects of cadmium on the proximal renal tubules

As it has already been mentioned, proximal tubular cells are the main site of cadmium deposition (8). It is deemed that free Cd^{2+} has the main role in the damage, while metallothioneins have the protective role and that it comes to the damage when the capacity of kidneys to produce metallothioneins is exhausted. Studies conducted on mice that cannot synthesize metallothioneins and mice that can synthesize them show that the first group of mice is more sensitive to the toxic effect of cadmium, and since there are great variations regarding the expression of metallothionein in people, some could be predisposed to the toxic effect induced by cadmium (13,20). However, certain studies suggest that metallothioneins to the greatest extent have the protective effect on the liver cells in comparison to the renal cells (20).

Most substances from primary urine are reabsorbed in proximal tubular cells in kidneys mainly through secondary active transport – co-transport with sodium (19). Therefore, it is not surprising that great number of studies examine the influence of cadmium on the transport processes in renal proximal tubular cells. Since the majority of substances are transported according to the electrochemical gradient of sodium, changes of membrane potential influence, to the greatest extent, transport processes (21). At the basolateral membrane of proximal tubular cells, there are potassium channels, through which K^+ is transported from the cell into interstitium, down the concentration gradient, thus enabling the hyperpolarization of basolateral membrane. Basolateral and luminal membranes are electrically connected, and therefore, this polarization is transferred to luminal membrane, as well. Hyperpolarization of luminal membrane is important because it provides electrical gradient – driving force for sodium to enter the cell.

Peritubular perfusion with micromolecular concentrations of cadmium (Cd^{2+}) leads to the sustained, reversible hyperpolarization of basolateral, and later luminal membrane. The hyperpolarization of membrane occurs most probably due to the binding of cadmium to potassium channels on the basolateral membrane, which leads to the conformation changes of channels and therefore, potassium moves out

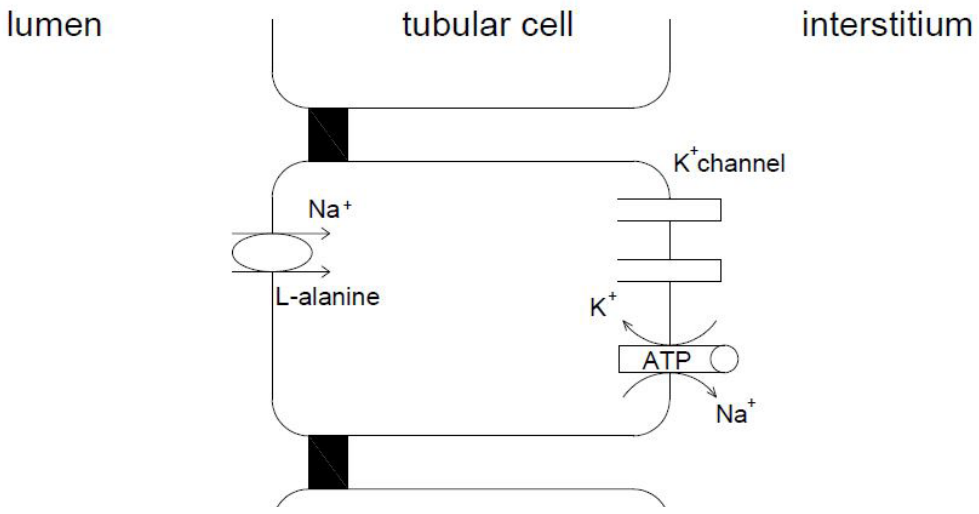
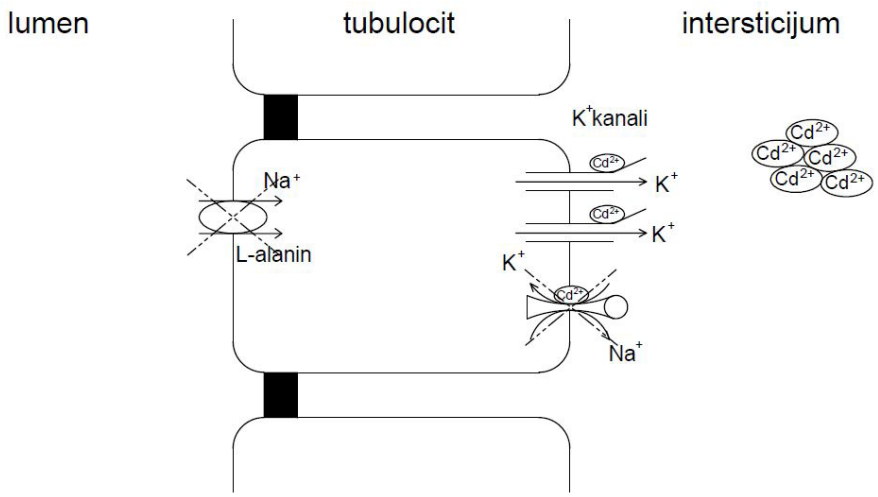


Figure 1. Scheme of transport processes in proximal tubular cells



Slika 2. Shematski prikaz efekata kadmijuma na transportne procese u proksimalnim tubulskim ćelijama

anina putem ovog transportera. Sa druge strane, izlaganje luminalne membrane mikromolarnim koncentracijama kadmijuma ne dovodi do smanjenja ovog kotransporta, odnosno, kadmijum nema direktno inhibitorno dejstvo na rad Na^+/L-alanin kotransportera (25). Ovakvi rezultati sugerišu da kadmijum pored uticaja na kalijumske kanale na bazolateralnoj membrani, verovatno stupa u interakciju i sa sulfhidrilnim grupama drugih proteina bazolateralne membrane. Moguće objašnjenje je da se kadmijum vezuje za sulfhidrilne grupe Na^+/K^+ ATP-aze, dovodi do njenih konformacionih promena usled čega se smanjuje njena aktivnost. Natrijum se zadržava u ćeliji, smanjuje se njegov koncentracioni gradijent te se posledično smanjuje transport preko Na^+/L-alanin kotransportera (27) (slika 2).

Iako glavno mesto u oštećenju transportnih procesa u bubrezima zauzima jonizovani kadmijum, postoje studije koje su pokazale da i kompleks Cd-MT dovodi do smanjenja transporta preko Na^+/L-alanin transportera i to direktnom inhibicijom samog transportera, ali i indirektno smanjenjem aktivnosti Na^+/K^+ ATP-aze na bazolateralnoj membrani. Ovaj kompleks takođe direktno deluje i na Na^+/Glu kotransporter smanjujući njegovu aktivnost (28).

Osim što dovodi do hiperpolarizacije bazolateralne membrane, peritubularna izloženost mikromolarnim koncentracijama kadmijuma (Cd^{2+}) smanjuje brzinu spore repolarizacije luminalne membrane. Do faze spore repolarizacije dolazi usled aktivacije kalijumskih kanala i kretanja kalijuma iz ćelije niz gradijent koncentracije. Moguće objašnjenje nalazi se u tome da kadmijum izaziva konformacione promene kalijumskih kanala na bazolateralnoj membrani usled čega kalijum pojačano izlazi iz ćelije i peritubularna koncentracija kalijuma se povećava (25,26). Zbog postojanja kružnog kola, kalijum prolazi kroz paracelularni šant te se njegova koncentracija povećava i sa luminalne strane (29). Ovo dovodi do smanjenja koncentracionog gradijenta i posledično smanjenja brzine protoka kalijuma iz ćelije što konačno rezultuje smanjenjem brzine spore repolarizacije (25,26).

Mali broj studija bavio se ispitivanjem direktnog efekta kadmijuma na endocitozno preuzimanje proteina u ćelijama proksimalnih tubula. U nedavnoj studiji, *Fujishiro* i saradnici su koristeći kultivisane bubrežne ćelije ispitivali efekte kadmijuma na endocitozno preuzimanje fluorescentno obeleženih albumina, β2 mikroglobulina, transferina i metalotioneina u proksimalne ćelije bubrega. Rezultati njihove studije pokazali su da se unos β2 mikroglobulina i metalotioneina smanjio nakon trodnevnog izlaganja subletalnim dozama kadmijuma, dok ovo izlaganje nije imalo uticaj na preuzimanje albumina i transferina. Jedno od objašnjenja ovakvih rezultata moglo bi biti *in vivo* zapažanje da je β2 mikroglobulin najosetljiviji marker oštećenja bubrežne tubularne reapsorpcije (30).

Zaključak

Brojne studije sprovedene u okviru humane populacije, ali i na različitim animalnim modelima imale su za cilj ispitivanje toksičnih efekata kadmijuma. U tim istraživanjima korišćene su različite doze izlaganja kadmijumu, od mikromolarnih do

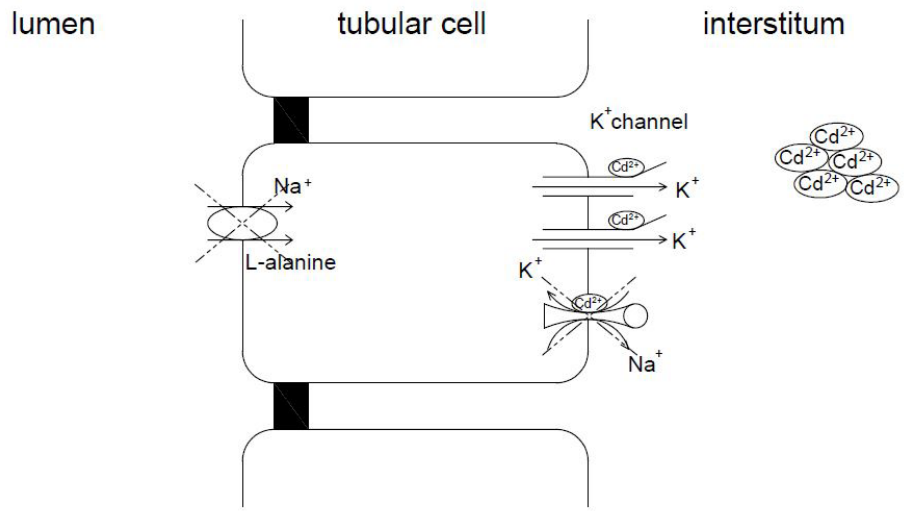


Figure 2. Scheme of the cadmium effects on transport processes in proximal tubular cells

of the cell increasingly (24-26) (Figure 2). It was shown that hyperpolarization occurred as a result of increased conductance of basolateral potassium channels when they were inhibited by non-specific barium that abolished hyperpolarization (25,26). However, this hyperpolarization was followed by the reduction in the amplitude of depolarization induced by the activation of Na^+/L-alanine co-transporter on the luminal membrane (27), which could point to the reduction in reabsorption of L-alanine with the help of this transporter. On the other hand, exposure of luminal membrane to micromolar concentrations of cadmium does not lead to the reduction in this co-transporter, that is, cadmium does not have a direct inhibiting effect on Na^+/L-alanine co-transporter (25). Such results suggest that cadmium, in addition to the potassium channels on the basolateral membrane, probably interacts with sulfhydryl groups of other proteins of basolateral membrane. A possible explanation is that cadmium is bound to sulfhydryl groups Na^+/K^+ ATPase, which leads to conformation changes due to which its activity is reduced. Sodium is maintained in the cell, its concentration gradient is reduced which consequentially reduces the transport through Na^+/L-alanine co-transporters (27) (Figure 2).

Although ionized cadmium takes the main place in the damage of transport processes in kidneys, there are studies which have shown that Cd-Mt complex leads to the reduction in transport through Na^+/L-alanine, that is, with the direct inhibition of the transporter, and indirectly by reducing the activities of Na^+/K^+ ATPase on the basolateral membrane. This complex also directly influences Na^+/GLU co-transporter by reducing its activity (28).

In addition to the fact that it leads to the hyperpolarization of basolateral membrane, peritubular exposure to micromolar concentrations of cadmium (Cd^+) reduces the speed of slow repolarization of luminal membrane. The phase of slow repolarization is induced by activation of potassium channels and movement of potassium out of the cell down the concentration gradient. A possible explanation may be that cadmium causes conformation changes of potassium channels on the basolateral membrane, due to which potassium moves out of the cell increasingly and therefore, peritubular concentration of potassium is increased (25,26). Due to the existence of circuit, potassium passes through the paracellular shunt and therefore, its concentration increases at the luminal side (29). This causes the reduction in the concentration gradient resulting in the decrease of the speed of potassium flow from the cell, which finally causes the reduction in the speed of slow repolarization (25,26).

Few studies have examined the direct effects of cadmium on endocytic uptake of proteins into proximal tubular cells. In a recent study, by using the cultivated renal cells, *Fujishiro* and associates have examined the effects of cadmium on endocytic uptakes of fluorescently marked albumin, ß2 microglobulin, transferrin and metallothionein into proximal renal cells. The results of their study have shown that the intake of ß2 microglobulin and metallothionein decreased after a three-day

milimolarnih. Studije izvedene na animalnim modelima po vrednosti dobijenih podataka, ne zaostaju za istraživanjima u humanoj populaciji, s obzirom da su korišćene životinjske vrste koje odlikuje velika strukturna i funkcionalna sličnost organa, u prvom redu bubrega, sa humanim bubregom, koji je jedan od glavnih meta deponovanja kadmijuma nakon izlaganja.

Kadmijum se deponuje u organizmu u najvećem procentu u ćelijama proksimalnih tubula bubrega gde direktno ili indirektno menja aktivnost ćelijskih transportnih sistema smanjujući reapsorpciju aminokiselina, glukoze, niskomolekularnih proteina. Peritubulska perfuzija ovih ćelija mikromolarnim koncentracijama Cd^{2+}, u *in vitro* uslovima, dovodi do održavane, reverzibilne hiperpolarizacije peritubulskog membranskog potencijala (PD) i povećava kalijumsku selektivnost njihove bazolateralne membrane. Takođe, akutno peritubulsko izlaganje, proksimalnih tubulskih ćelija, mikromolarnim koncentracijama Cd^{2+} izaziva smanjenje visine brze depolarizacije i brzine spore repolarizacije (PD), prilikom istovremene luminalne aplikacije L-alanina, u poređenju sa ovim parametrima pri izlaganju L-alanina u odsustvu Cd^{2+}. Obzirom da se radi o reverzibilnim efektima, oni se mogu smatrati posledicom vezivanja Cd^{2+} za sulfhidrilne (-SH) grupe proteina koji se nalaze u bazolateralnoj membrani ovih ćelija. Ovo smanjenje visine brze depolarizacije i brzine spore repolarizacije (PD), za posledicu može imati smanjenu reapsorpciju L-alanina, a to bi mogao biti rani znak oštećenja proksimalne tubulske ćelije prilikom akutnog izlaganja mikromolarnim koncentracijama kadmijuma.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

Literatura

1. WHO – World Health Organisation. Available at: www.who.int (18.05.2022.)
2. CDC – Center for Disease Control. Available at: www.cdc.gov (18.05.2022.)
3. Dunglison R, Dunglison R. Medical lexicon. Philadelphia: Henry C. Lea; 1874.
4. ATSDR - Agency for Toxic Substances and Disease Registry. Available at: www.atsdr.cdc.gov (18.05.2022.)
5. Rani A, Kumar A, Lal A, Pant M. Cellular mechanisms of cadmium-induced toxicity: a review. Int J Environ Health Res 2014; 24(4):378-99. doi: 10.1080/09603123.2013.835032
6. Ninkov MN. Intestinalni i sistemski imunski efekti oralnog unosa kadmijuma kod pacova [disertacija]. Beograd: Biološki fakultet Univerziteta u Beogradu; 2016.
7. Pešić BČ. Patofiziologija: mehanizmi poremećaja zdravlja, I deo. 1st ed. Belgrade: Medicinski fakultet Univerziteta, CIBID; 2015.
8. Vidaković A, Bulat P, Milačić S, Milovanović A, Pavlović M, Torbica N, et al. editors. Osnovi medicine rada. 1st ed. Belgrade: Medicinski fakultet Univerziteta, CIBID; 2014.
9. Ryu DY, Lee SJ, Park DW, Choi BS, Klaassen CD, Park JD. Dietary iron regulates intestinal cadmium absorption through iron transporters in rats. Toxicol Lett 2004; 152(1):19-25. doi: 10.1016/j.toxlet.2004.03.015
10. Zalups RK, Ahmad S. Molecular handling of cadmium in transporting epithelia. Toxicol Appl Pharmacol 2003; 186(3):163-88. doi: 10.1016/s0041-008x(02)00021-2
11. Li Y, Huang YS, He B, Liu R, Qu G, Yin Y, et al. Cadmium-binding proteins in human blood plasma. Ecotoxicol Environ Saf 2020; 188:109896. doi: 10.1016/j.ecoenv.2019.109896
12. Sabolić I, Breljak D, Skarica M, Herak-Kramberger CM. Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs. Biometals 2010; 23(5):897-926. doi: 10.1007/s10534-010-9351-z
13. Klaassen CD, Liu J, Diwan BA. Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 2009; 238(3):215-220. doi:10.1016/j.taap.2009.03.026
14. Jin T, Lu J, Nordberg M. Toxicokinetics and biochemistry of cadmium with special emphasis on the role of metallothionein. Neurotoxicology 1998; 19(4-5):529-35.
15. Yang H, Shu Y. Cadmium transporters in the kidney and cadmium-induced nephrotoxicity. Int J Mol Sci 2015; 16(1):1484-94. doi: 10.3390/ijms16011484
16. Swiergosz-Kowalewska R. Cadmium distribution and toxicity in tissues of small rodents. Microsc Res Tech 2001;55(3):208-22. doi: 10.1002/jemt.1171
17. Atanacković M et al. editors. Patologija. 3rd ed. Belgrade: Medicinski fakultet Univerziteta, CIBID; 2009.
18. Bumbaširević V, Lačković V, Milićević N, Mujović S, Obradović M, Pantić S et al., editors. Histologija. 2nd ed. Belgrade: Medicinski fakultet Univerziteta, CIBID; 2007.
19. Kojić Z, Stanojlović O, editors. Fiziologija za studente medicine: odabrana poglavlja, II deo. 2nd ed. Belgrade: Medicinski fakultet Univerziteta, CIBID 2014.
20. Liu J, Liu Y, Michalska AE, Choo KH, Klaassen CD. Metallothionein plays less of a protective role in cadmium-metallothionein-induced nephrotoxicity than in cadmium chloride-induced hepatotoxicity. J Pharmacol Exp Ther 1996; 276(3):1216-23.
21. Zelikovic I, Chesney RW. Sodium-coupled amino acid transport in renal tubule. Kidney Int 1989; 36(3):351-9. doi: 10.1038/ki.1989.203
22. Čemerikić AD. Elektrofiziologija bubrežnog transporta. In: Mujović MV, Čemerikić AD, editors. Fiziologija bubrega. Beograd: Naučna knjiga, 1987: 15-36.

exposure to sublethal doses of cadmium, while this exposure did not have effects on the uptake of albumin and transferrin. One of the explanations of such results could be in vivo observation that ß2 microglobulin is the most sensitive marker of damage of renal tubular reabsorption (30).

Conclusion

The aim of numerous studies conducted within human populations and on different animal models was to examine the toxic effects of cadmium. In these studies, different doses of exposure to cadmium were used, from micromolar to millimolar. Studies conducted on animal models according to the values of obtained data do not lag behind studies of human populations, considering the fact that they used animal species characterized by great structural and functional similarity between organs, first of all, kidneys and human kidneys, which is one of the main targets of cadmium deposits after exposure.

Cadmium is deposited in the body to the greatest extent in proximal tubular cells, where it directly or indirectly changes the activity of transport systems of cells by reducing the reabsorption of amino acids, glucose and low-molecular-weight proteins. Peritubular perfusion of these cells with micromolar concentrations of Cd^{2+} in in vitro conditions leads to a sustained, reversible hyperpolarization of peritubular membrane potential (PD) and increases potassium selectivity of their basolateral membrane. Also, acute peritubular exposure of proximal tubular cells to micromolar concentrations of Cd^{2+} causes the reduction in the amplitude of fast depolarization and speed of slow repolarization (PD), during the simultaneous luminal application of L-alanine in comparison to these parameters during the exposure to L-alanine in the absence of Cd^{2+}. Considering the fact that these effects are reversible, they may be deemed to be the result of binding of Cd^{2+} to sulfhydryl groups of proteins in the basolateral membrane of these cells. This reduction in the amplitude of fast depolarization and the speed of slow repolarization (PD) can cause the decreased reabsorption of L-alanine, and that could be the early sign of damage of proximal tubular cells during acute exposure to micromolar concentrations of cadmium.

Competing interests

The author declares no competing interests.

Literature

1. WHO – World Health Organisation. Available at: www.who.int (18.05.2022.)
2. CDC – Center for Disease Control. Available at: www.cdc.gov (18.05.2022.)
3. Dunglison R, Dunglison R. Medical lexicon. Philadelphia: Henry C. Lea; 1874.
4. ATSDR - Agency for Toxic Substances and Disease Registry. Available at: www.atsdr.cdc.gov (18.05.2022.)
5. Rani A, Kumar A, Lal A, Pant M. Cellular mechanisms of cadmium-induced toxicity: a review. Int J Environ Health Res 2014; 24(4):378-99. doi: 10.1080/09603123.2013.835032
6. Ninkov MN. Intestinalni i sistemski imunski efekti oralnog unosa kadmijuma kod pacova [disertacija]. Beograd: Biološki fakultet Univerziteta u Beogradu; 2016.
7. Pešić BČ. Patofiziologija: mehanizmi poremećaja zdravlja, I deo. 1st ed. Belgrade: Medicinski fakultet Univerziteta, CIBID; 2015.
8. Vidaković A, Bulat P, Milačić S, Milovanović A, Pavlović M, Torbica N, et al. editors. Osnovi medicine rada. 1st ed. Belgrade: Medicinski fakultet Univerziteta, CIBID; 2014.
9. Ryu DY, Lee SJ, Park DW, Choi BS, Klaassen CD, Park JD. Dietary iron regulates intestinal cadmium absorption through iron transporters in rats. Toxicol Lett 2004; 152(1):19-25. doi: 10.1016/j.toxlet.2004.03.015
10. Zalups RK, Ahmad S. Molecular handling of cadmium in transporting epithelia. Toxicol Appl Pharmacol 2003; 186(3):163-88. doi: 10.1016/s0041-008x(02)00021-2
11. Li Y, Huang YS, He B, Liu R, Qu G, Yin Y, et al. Cadmium-binding proteins in human blood plasma. Ecotoxicol Environ Saf 2020; 188:109896. doi: 10.1016/j.ecoenv.2019.109896
12. Sabolić I, Breljak D, Skarica M, Herak-Kramberger CM. Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs. Biometals 2010; 23(5):897-926. doi: 10.1007/s10534-010-9351-z
13. Klaassen CD, Liu J, Diwan BA. Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 2009; 238(3):215-220. doi:10.1016/j.taap.2009.03.026
14. Jin T, Lu J, Nordberg M. Toxicokinetics and biochemistry of cadmium with special emphasis on the role of metallothionein. Neurotoxicology 1998; 19(4-5):529-35.
15. Yang H, Shu Y. Cadmium transporters in the kidney and cadmium-induced nephrotoxicity. Int J Mol Sci 2015; 16(1):1484-94. doi: 10.3390/ijms16011484
16. Swiergosz-Kowalewska R. Cadmium distribution and toxicity in tissues of small rodents. Microsc Res Tech 2001;55(3):208-22. doi: 10.1002/jemt.1171
17. Atanacković M et al. editors. Patologija. 3rd ed. Belgrade: Medicinski fakultet Univerziteta, CIBID; 2009.

23. Lang F, Messner G, Rehwald W. Electrophysiology of sodium-coupled transport in proximal renal tubules. Am J Physiol 1986; 250(6Pt2):F953-62. doi: 10.1152/ajprenal.1986.250.6.F953
24. Li S, Yu J, Zhu M, Zhao F, Luan S. Cadmium impairs ion homeostasis by altering K^+ and Ca^{2+} channel activities in rice root hair cells. Plant Cell Environ 2012; 35(11):1998-2013. doi: 10.1111/j.1365-3040.2012.02532.x
25. Nešović-Ostojić J, Čemerikić D, Dragović S, Milovanović A, Milovanović J. Low micromolar concentrations of cadmium and mercury ions activate peritubular membrane K+ conductance in proximal tubular cells of frog kidney. Comp Biochem Physiol A Mol Integr Physiol 2008; 149(3):267-74. doi: 10.1016/j.cbpa.2007.12.006
26. Nešović J, Cemerikić D. Characterization of luminal and peritubular membrane K^+ selectivity in proximal tubular cells of frog kidney. Ann N Y Acad Sci 2005; 1048:441-4. doi: 10.1196/annals.1342.059
27. Nešovic-Ostojić J, Kovačević S, Spasić S, Lopičić S, Todorović J, Dinčić M, et al. Modulation of luminal L-alanine transport in proximal tubular cells of frog kidney induced by low micromolar Cd2+ concentration. Comp Biochem Physiol C Toxicol Pharmacol 2019; 216:38-42. doi: 10.1016/j.cbpc.2018.11.007
28. Tsuruoka S, Sugimoto K, Muto S, Nomiyama K, Fujimura A, Imai M. Acute effect of cadmium-metallothionein on glucose and amino acid transport across the apical membrane of the rabbit proximal tubule perfused in vitro. J Pharmacol Exp Ther 2000; 292(2):769-77.
29. Frömter E, Diamond J. Route of passive ion permeation in epithelia. Nat New Biol 1972; 235(53):9-13. doi: 10.1038/newbio235009a0
30. Fujishiro H, Yamamoto H, Otera N, Oka N, Jinno M, Himeno S. In vitro evaluation of the effects of cadmium on endocytic uptakes of proteins into cultured proximal tubule epithelial cells. Toxics 2020; 8(2):24. doi: 10.3390/toxics8020024

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18. Bumbaširević V, Lačković V, Milićević N, Mujović S, Obradović M, Pantić S et al., editors. Histologija. 2nd ed. Belgrade: Medicinski fakultet Univerziteta, CIBID; 2007.
19. Kojić Z, Stanojlović O, editors. Fiziologija za studente medicine: odabrana poglavlja, II deo. 2nd ed. Belgrade: Medicinski fakultet Univerziteta, CIBID 2014.
20. Liu J, Liu Y, Michalska AE, Choo KH, Klaassen CD. Metallothionein plays less of a protective role in cadmium-metallothionein-induced nephrotoxicity than in cadmium chloride-induced hepatotoxicity. J Pharmacol Exp Ther 1996; 276(3):1216-23.
21. Zelikovic I, Chesney RW. Sodium-coupled amino acid transport in renal tubule. Kidney Int 1989; 36(3):351-9. doi: 10.1038/ki.1989.203
22. Čemerikić AD. Elektrofiziologija bubrežnog transporta. In: Mujović MV, Čemerikić AD, editors. Fiziologija bubrega. Beograd: Naučna knjiga, 1987: 15-36.
23. Lang F, Messner G, Rehwald W. Electrophysiology of sodium-coupled transport in proximal renal tubules. Am J Physiol 1986; 250(6Pt2):F953-62. doi: 10.1152/ajprenal.1986.250.6.F953
24. Li S, Yu J, Zhu M, Zhao F, Luan S. Cadmium impairs ion homeostasis by altering K^+ and Ca^{2+} channel activities in rice root hair cells. Plant Cell Environ 2012; 35(11):1998-2013. doi: 10.1111/j.1365-3040.2012.02532.x
25. Nešović-Ostojić J, Čemerikić D, Dragović S, Milovanović A, Milovanović J. Low micromolar concentrations of cadmium and mercury ions activate peritubular membrane K^+ conductance in proximal tubular cells of frog kidney. Comp Biochem Physiol A Mol Integr Physiol 2008; 149(3):267-74. doi: 10.1016/j.cbpa.2007.12.006
26. Nešović J, Cemerikić D. Characterization of luminal and peritubular membrane K+ selectivity in proximal tubular cells of frog kidney. Ann N Y Acad Sci 2005; 1048:441-4. doi: 10.1196/annals.1342.059
27. Nešovic-Ostojić J, Kovačević S, Spasić S, Lopičić S, Todorović J, Dinčić M, et al. Modulation of luminal L-alanine transport in proximal tubular cells of frog kidney induced by low micromolar Cd2+ concentration. Comp Biochem Physiol C Toxicol Pharmacol 2019; 216:38-42. doi: 10.1016/j.cbpc.2018.11.007
28. Tsuruoka S, Sugimoto K, Muto S, Nomiyama K, Fujimura A, Imai M. Acute effect of cadmium-metallothionein on glucose and amino acid transport across the apical membrane of the rabbit proximal tubule perfused in vitro. J Pharmacol Exp Ther 2000; 292(2):769-77.
29. Frömter E, Diamond J. Route of passive ion permeation in epithelia. Nat New Biol 1972; 235(53):9-13. doi: 10.1038/newbio235009a0
30. Fujishiro H, Yamamoto H, Otera N, Oka N, Jinno M, Himeno S. In vitro evaluation of the effects of cadmium on endocytic uptakes of proteins into cultured proximal tubule epithelial cells. Toxics 2020; 8(2):24. doi: 10.3390/toxics8020024

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