EFFECT OF ACUTE RENAL FAILURE ON KIDNEY AMIDINOTRANSFERASE ACTIVITY

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L-Arginine-:glycine amidinotransferase (EC 2.1.4.1) catalyzes the transfer of an amidino group from arginine to glycine to form guanidinoacetate, precursor in creatine synthesis. The kidneys are major site of the creatine synthesis and primary target organs for mercury toxicity. In evaluation of molecular mechanisms of mercury chloride intoxication relating to creatine metabolism we have investigated the enzyme activity in kidney tissue after mercury chloride administration. Acute renal failure was induced by i.p administration of mercury chloride in a dose of 3 mg/kg to male Spraque Dawley rats weighing about 200 g. The results of our study indicate an acute renal failure 24 hours after mercury chloride administration. Urea and creatinine levels in blood plasma were significantly elevated compared to control group ($p<0.001$). Amidinotransferase activity in kidney tissue was depressed, while, in plasma of intoxicated rats activity of enzyme was increased ($p<0.001$). The obtained results indicate that mercury chloride has strong nephrotoxic effect. Depressed amidinotransferase activity and decreased production of guanidinoacetate, initial product in creatine synthesis, may be implicated in neurotoxicity, cardiotoxicity and muscle damage in mercury intoxication, because creatine and its phosphorylated form creatine phosphate play an important role in the energy metabolism. *Acta Medica Medianae 2004; 43(2):5-8.*

Key words: *mercury chloride, kidney, amidinotransferase, urea, creatinine, creatine*

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Introduction

The kidneys are the target for numerous toxicants including environmental chemical substances. Humans and animals are exposed to numerous chemical forms of mercury: elemental mercury vapor (Hg°), inorganic mercurious (Hg^{+}) and mercuric (Hg^{2+}) compounds, and organic mercuric compounds (R-Hg^{+} or R-Hg-R). All forms of mercury have toxic effects (1). Many inorganic compounds of mercury are used in agriculture (pesticides, fungicides), medicine (as a desinfectants, dental amalgam fillings, vaccines) industrial manufacture (fluorescent lamps, batteries, thermostats, thermometers).

Mercury chloride ($HgCl_2$) is inorganic mercury compound with ionic mercury. Intoxication with mercury occurs through environmental, occupational or accidental exposure. Fish and fungicides are the main source of methyl mercury. Babies are exposed to ethyl mercury through vaccination and adults through dental amalgam filings (2,3).

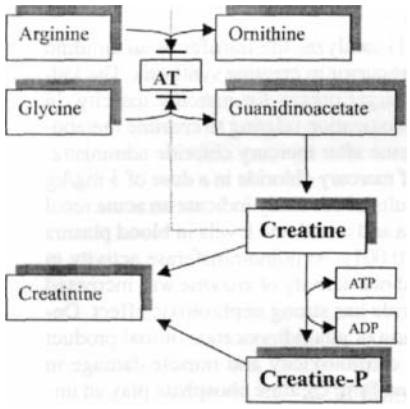
Acute exposure to mercury may include damage of the kidney, gastrointestinal, nervous and cardiovascular system. Chronic exposure leads to neurodegenerative disorders like Alzheimer's diseases, Parkinson's diseases, autism, irnmunomodulation, dermatitis, reproductive failure, chronic renal failure, cardiovascular and liver damage and carcinogenesis. Manifestations of toxicity include: headache, tremor, impaired coordination, abdominal cramps, diarrhea, dermatitis, polyneuropathy, proteinuria, and hepatic dysfunction (4,5,6,7, 8,9).

Mercury is accumulated and expresses toxicity primary to the kidney (9,10). Acute renal failure (ARF) is a dramatical clinical syndrome. It develops after acute exposure to high doses of mercury chloride and is frequently fatal. Toxic effects caused by mercury itself and / or by numerous secondary reactions in the body, reflect many biochemical parameters in blood and kidney tissue.

Kidney has an important role in metabolism of arginine. Among other roles in the kidney, arginine is also substrate for the synthesis of guanidinoacetate, precursor for creatine synthesis. This reaction is the first and limiting reaction in creatine synthesis catalyzed by amidinotransfrase (Transamidinase, L-arginine-glycine transamidinase, EC 2.1.4.1). Amidinotransferase (AT) is predominantly located in proximal tubules of the kidneys. Guanidinoacetate in the second reaction, mainly in the liver, was methylated by the activity of guanidinoacetate methyltransferase which transfers methyl group from S-adenosyl-methionine to guanidinoacetate forming creatine. Creatine undergoes

to creatirie phosphate by creatine kinase activity in muscle, heart, brain and tissues which possess high creatine kinase activity. The role of creatine phosphate in these tissues is to provide a high energy phosphate which is important for ATP synthesis or ATP utilization. In the state of constant functional activity, creatine and creatine phosphate foni. creatinine by nonenzymatic cyclization. Creatinine, as terminal product, vvas excreted by the kidney in urine (11,12) (Figure l).

Proximal tubules are the most vulnerable part of the nephron to toxic effect of mercury (13,14).



Figurel. Creatine synthesis

The aim

The mechanisms by which mercury chloride induces renal injury are not completely understood. Therefore, we studied renal arginine metabolism relating to creatine metabolism in rat model nephrotoxicity induced by mercury chloride. We analyzed the levels of urea, creatinine and transamidinase activity as possible biochemical markers of kidney tissue.

Materials and methods

Male Spraque Dawley rats vveighing about 200 g. were used in the experiment. Acute renal failure was induced by i.p administration of mercury chloride in a dose of 3 mg/kg. Control group of animals was treated with equal volume of saline. Urea and creatinine levels in blood plasma were measured 24 hours after the induction of acute uremic syndrome by standard biochemical analysis. Transamidinase activity in kidney tissue and blood plasma were measured according to the method Van Pilsum et al. (15) with minor modification. Enzyme activity was measured by determination of the amounts of formed ornithine by ninhydrine color reaction and expressed in units on mg of tissue proteins. Tissue level of proteins vvas determined by the method of Lowry et al. (16).

Results

Data on renal function vvere shovvn in Table 1.

Plasma levels of urea and creatinine were significantly increased in $HgCl_2$ intoxicated rats compared to the control group ($p < 0.001$). These animals show higher levels of plasma transamidinase activity compared to control £roi'p ($p< 0.001$) (Figure 2).

Table l. Plasma levels of urea and creatinine

Investigated groups	Urea (mmol/L)	Creatinine (mmol/L)
Control	7.9 ±0.5	40.34 ± 8.3
$HgCl_2$	22.7 ± 1.6 ***	288128.4***

*p<0.001

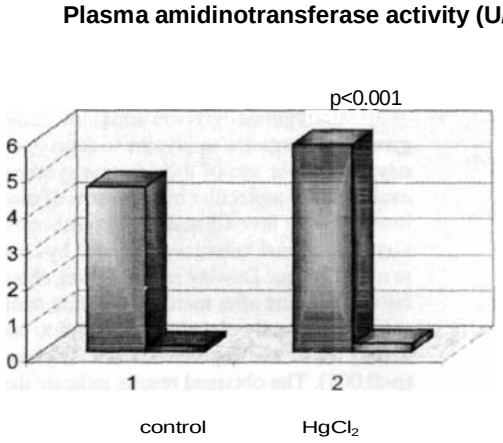


Figure 2. Plasma activitv of amidinotransferase. Results are expressed as means +/-SD

Kidney tissue level of transamidinase was significantly decreased in animals treated vvith mercury chloride ($p<0.001$) (Figure 3).

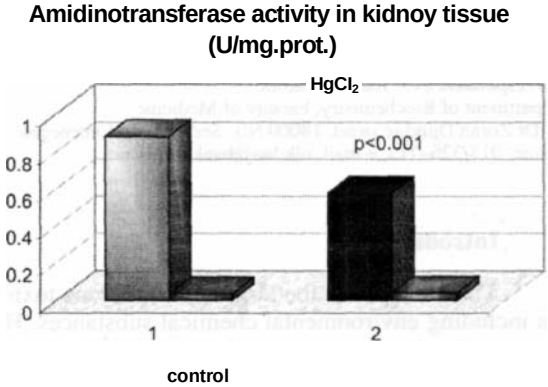


Figure 3. Kidnev amidinotrasferase activitv. Results are expressed as means +/-SD

Discussion

Creatinine and urea are used still today as clinical makers for renal function. Significant elevation of urea and creatinine levels in blood plasma in rats treated vvith mercury chloride indicate acule renal insufficiency. Factors contributing to the sensitivity of the kidney to mercury include large blood flovv, the presence of xenobiotic transporters and metabolizing enzymes (10,17). Decrease in glomerular filtration rate, tubular collapse, and marked structural and functional tubular abnormalities vvere reported from Conger and Falk (18).

Decrease transamidinase in kidney tissue may be a result of enzyme inhibition and/or ćeli necrosis. Inhi-

bition of enzyme may be caused by mercury chloride and hyperonithinemia. Hyperornithinemia results in enzyme inhibition because ornithine is strong competitive inhibitor of amidinotransferase (19).

Renal accumulation of mercury is caused by the transformation of mercury to mercuric ion. Interactions with sulfhydryl groups in molecules of albumin, metallothionein, glutathione, and cysteine have been implicated in mechanisms involved in the proximal tubular uptake, accumulation, transport, and toxicity of mercuric ions (20,21,22,23). Amidinotransferase is thiol enzyme with Cys407 in active site residue (24). Binding of $HgCl_2$ vvith SH group of enzyme leads to inhibition of enzyme activity (25). Transamidinase is predominantly located in proximal tubules of the kidneys where $HgCl_2$ induce severe damage. Necrosis of proximal tubular cells leads to release of enzyme in blood. Elevation of plasma enzyme was also seen in various kidney diseases (26).

One of pathogenetic mechanisms of acute renal failure is rhabdomyolysis, elevation of serum concentration of aldolase and creatine phosphokinase, and the presence of pigment granular casts and myoglobin in the urine (27). Low level of amidinotransferase activity in kidney tissue may be due to high levels of blood and kidney creatine content which is released as a result of catabolic effect of adrenal steroids on muscle mass (28). Creatine inhibits ovvn synthesis by inhibition of amidinotransferase and decrease formation of guanidinotransferase. Creatine affects enzyme activity by changing its rate of synthesis at a pretranslational šlep and represents an example of end-product repression. Feedback repression of amidinotransferase by creatine is expressed in kidney and pancreas (11,12,29,30,31).

Creatine movement through ćeli membranes requires creatine transporters which are Na^+,Cl~ dependent. Creatine transporters contain a cysteine residue, cysteine 144, in the third transmembrane domain of the creatine transporter. It is located close to a substrate-binding site (32). Due to a vvell-knovvn high affinity of mercury to SH-groups there is a possibility that binding of mercury vvith creatine transporters alters moving creatine through ćeli membranes. Therefore, recent researches point disorders of creatine metabolism on the level of creatine synthesis and creatine transport (33, 34, 35).

The main function of creatine is in ATP production through its involvement in phosphocreatine energy system through phosphocreatinine shuttle. In the reversible reaction catalyzed by creatine kinase, creatine and ATP form phosphocreatine and adenosine diphosphate (ADP). This energy shuttle uses phosphocreatine to carry energy between sites of production and sites of utilization (36, 37).

Conclusion

In acute renal failure induced by mercury chloride kidney tissue level of transamidinase activity was decreased. Results of our study indicate on important mechanism in nephrotoxicity of mercury chloride, which could be involved in developing other organ and system failure. Decrease of creatine and creatine phosphate in brain, muscle and heart, as a consequence of transamidinase inhibition, has influence on energetic metabolism in these tissues and could lead to neurotoxicity, cardiotoxicity or muscle damage. Results of our study indicate important role of creatine in mercury intoxication and possible beneficial effect of creatine supplementation in mercury chloride intoxication.

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AKTIVNOST AMIDINOTRANSFERAZE BUBREGA U AKUTNOJ BUBREŽNOJ INSUFICIJENCIJI

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Amidinotransferaza (L-arginin-glicin amidinotransferaza, transamidinaza, EC 2.1.4.1) je enzim koji katalizuje prenos amidino grupe sa arginina na glicin pri cemu nastaje glikociamin, prekursor u sintezi kreatina. Bubrezi su glavno Čicsto sinteze kreatina i primarni target organi za toksicne efekte žive. U cilju ispitivanja molekulskih mehanizama toksičnosti merkuri hlorida ispitivana je aktivnost enzima u plazmi i bubrežnom homogenatu. Akutna bubrežna insuficijencija je izazvana na muskim Spraque Dawley pacovima intraperitonealnom primenom merkuri hlorida u dozi od 3 mg/kg. Rezultati istraživanja pokazuju da se nakon 24 časa od primene žive razvijaju simptomi akutne bubrezne insuficijencije; u odnosu na kontrolnu grupu životinja dolazi do signifikantnog porasta uree i kreatinina u plazmi ($p<0.001$). Aktivnost amidinotransferaze u homogenatu bubrežnog tkiva se smanjuje ($p<0.001$), dok se u plazmi enzimska aktivnost povećava ($p<0.001$). Dobijeni rezultati ukazuju na izrazito nefrotoksično dejstvo merkuri hlorida. Smanjenje aktivnosti amidinotransferaze u bubrezima i smanjena sinteza glikociamina, prekursora kreatina, može biti vazan mehanizam u pojavi neurotoksičnosti, kardiotoksičnosti i oštećenja mišića u intoksikaciji živom jer kreatin i njegova fosforilisana forma, kreatin fosfat, imaju važnu ulogu u energetskom metabolizmu. *Acta Medica Medianae 2004; 43(2): 5-8.*

***Klčne** reči: merkuri hlorid, huhreg, amidinotransferaza, urea, kreatinin, kreatin*