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The role of L-arginine in toxic liver failure: interrelation of arginase, polyamine catabolic enzymes and nitric oxide synthase

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Summary. The existing interrelation in metabolic pathways of L-arginine to polyamines, nitric oxide (NO) and urea synthesis could be affected in sepsis, inflammation, intoxication and other conditions. The role of polyamines and NO in the toxic effect of mercury chloride on rat liver function was studied. Administration of mercury chloride for 24 h led to significantly elevated plasma activities of Alanine transaminase (ALT) and Aspartate transaminase (AST). Malondyaldehyde (MDA) levels were unaffected ($p > 0.05$) and arginase activity was significantly decreased ($p < 0.05$) while nitrate/nitrite production was significantly elevated ($p < 0.001$) in liver tissue. Polyamine oxidase (PAO) and diamine oxidase (DAO) activities, enzymes involved in catabolism of polyamines, were decreased. L-arginine supplementation to intoxicated rats potentiated the effect of mercury chloride on NO production and it was ineffective on arginase activity.

Results obtained in this study show that mercury chloride-induced toxicity leads to abnormally high levels of ALT and AST that may indicate liver damage with the involvement of polyamine catabolic enzymes and NO.

Keywords: Arginase – Nitric oxide synthase – Polyamine oxidase – Diamine oxidase – Mercury chloride – Liver

Introduction

Exposure to numerous chemical forms of mercury, includes pure element, mercury vapor, inorganic compounds such as mercury chloride, and organic mercury (Fitzgerald and Clarkson, 1991). All forms of mercury cause toxic effects in a number of tissues and organ depending on the chemical form of mercury. Mercury has been used commercially and medically as a common constituent of many medical instruments such as thermometers, barometers and blood-pressure cuffs. It is constituent in batteries, switches, and fluorescent lamps. For mankind, major sources to exposure are dental amalgams, fish consumption, and vaccines. Clinical signs of toxicity dependent on doses and duration of exposure. Dental amalgam emits mercury vapor that is inhaled and absorbed. The amalgam consists of approximately 50 percent mercury. Long-term exposure to low concentrations of mercury vapor from amalgams may be a reason for developing of neurodegenerative diseases such as amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease and Parkinson's disease. Fish and fungicides are the main sources of methyl mercury (Molin et al., 1990; Clarkson et al., 2003; Block et al., 2004). Acrodynia, a childhood disease, is caused by mercuric and mercurous salts (Warkany and Hubbard, 1953). Babies are exposed to ethyl mercury, which is the active ingredient of the preservative thimerosal, through vaccination (Ball et al., 2001).

Different mechanisms are involved in the toxic effect of mercury chloride that leads to changes in cell functions. It occurs as results of direct toxic effect of mercury or its ions binding to thiol groups in some proteins, glutathione and enzymes (Johnson, 1982; Lash et al., 1998).

Arginine is a common substrate for the synthesis of urea, nitric oxide, agmatine, polyamines, creatine, proline and glutamate. L-ornithine, as a product of arginase activity, is necessary for the production of polyamines and proline (Wu and Morris, 1998). Arginine is converted to urea and ornithine by catalytic activity arginase. Arginase activity is linked with cell growth and connective tissue formation, which is related with polyamines and proline and in ammonia detoxication. Two separate isozymes of the enzyme arginase exist: type I is found in the liver and contributes the vast majority of

hepatic arginase activity, while type II is inducible and found in extrahepatic tissues.

Polyamines (putrescine, spermidine and spermine) are polycationic compounds derived from ornithine by activity of ornithine decarboxylase (ODC) and catabolized by DAO and PAO. Polyamines are essential to the growth and proliferation of mammalian cells. A lot of cellular functions of polyamines are still unknown (Pegg and McCann, 1982; Wallance et al., 2003).

Nitric oxide (NO) is the metabolite of L-arginine in the reaction catalyzed by nitric oxide synthase activity (NOS). This is a simple short-living molecule with different cell functions. NO is a highly reactive vasodilator, neuromodulator, imunomodulator (Wu and Morris, 1998; Ignarro et al., 2001). The aim of this study is the investigation of the role of polyamines and NO, metabolites of L-arginine, on liver function in acute toxicity induced by mercury chloride. In the evaluation of possible interrelation among the enzymes involved in the metabolism of L-arginine we have focused our attention to arginase, NOS, and polyamine catabolic enzymes, PAO and DAO activities. To study this relationship between polyamine and L-arginine metabolites, we checked the liver function indexes, ALT and AST, which reflect the severity of hepatocellular damage (Bass, 2003).

Material and methods

Experiments were performed on male Sprague Dawley rats weighing about 250 g. The animals were divided into 4 groups: 1 – control, treated with saline, 2 – mercury chloride treated group (3 mg/kg intraperitoneally), 3 – treated with L-arginine (250 mg/kg intraperitoneally) and 4 – treated with arginine one hour before mercury chloride in same doses. Animals were killed 24 h after mercury chloride administration. Blood plasma and liver homogenate were prepared and stored on $-70\,^{\circ}C$. In evaluation of liver function we measured plasma activities of ALT and AST by standard biochemical analysis. Lipid peroxidation level, as MDA was evaluated in liver tissue homogenate by thiobarbituric acid reaction (Stroev and Makarova, 1989). Arginase activity was assayed on the basis of the release of ornithine detected by ninhydrine color reaction (Porembska and Kedra, 1975). PAO and DAO were measured in liver homogenate according to the method of Bachrach and Reches (1966). Tissue protein level was determined according to Lowry et al. (1951). NOS was determined as nitrate/nitrite levels by the Griess reaction (Cortas and Wakid, 1990).

Statistical significance among groups was determined by Student-t test. $P < 0.05$ was used as statistically significant.

Results

Since liver enzymes, ALT and AST were elevated in blood plasma of mercury chloride-treated rats ($P < 0.001$) (Table 1), our finding shows that administration of mercury chloride leads to liver damage.

The levels of lipid peroxidation in liver, expressed as nmoles/mg of plasma proteins of MDA are shown in Table 2. There is no statistical significance for MDA level among the four experimental groups ($p > 0.05$).

Table 1. Alanine transaminase (ALT) and Aspartate transaminase (AST) activity in blood plasma

Treatments	ALT (U/L)	AST (U/L)
Control	34.5 ± 8.0	103 ± 17
$HgCl_2$	$127.5 \pm 21.1^{***}$	$266.5 \pm 13^{**}$
L-arginine	67.3 ± 4.2	112 ± 04
$HgCl_2$ + L-arginine	$118.80 \pm 8.0^{***}$	$405 \pm 30^{***}$

** $p < 0.05$
*** $p < 001$

Table 2. MDA levels in liver tissue

Treatments	MDA levels in liver (nmol/mg · protein)
Control	2.15 ± 0.08
$HgCl_2$	2.12 ± 0.085
L-arginine	2.05 ± 0.07
$HgCl_2$ + L-arginine	2.18 ± 0.065

On the contrary, liver arginase activity in mercury chloride-treated rat was significantly ($p < 0.05$) decreased (Fig. 1, group 2).

Nitrate and nitrite levels were significantlly elevated ($p < 0.001$), in treated rats compared to control (Fig. 2, group 2). Treatment with arginine one hour before mercury chloride shows that L-arginine administration to intoxicated rats is ineffective (Fig. 1, group 4) but probably

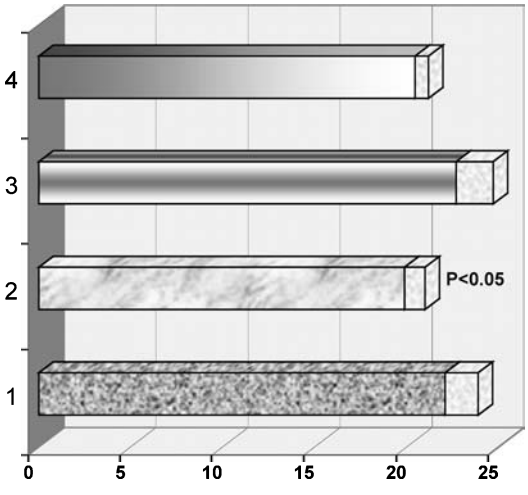

Fig. 1. Activity of liver arginase expressed as μmoles/mg of proteins: ***1*** Control; ***2*** animal treated with $HgCl_2$ (3 mg/kg itraperitoneally); ***3*** animal pretreated with L-arginine (250 mg/kg intraperitoneally); ***4*** animal pretreated with L-arginine (250 mg/kg intraperitoneally) and after 1 h treated with $HgCl_2$ (3 mg/kg intraperitoneally). Data are the mean of 4 experiments ± S.D

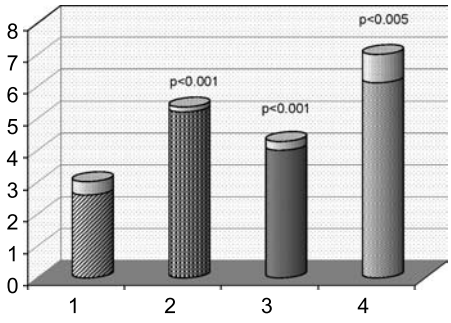

Fig. 2. Nitrate/nitrite levels in liver tissue expressed as μmoles/mg of proteins: ***1*** Control; ***2*** animal treated with $HgCl_2$ (3 mg/kg intraperitoneally); ***3*** animal pretreated with L-arginine (250 mg/kg intraperitoneally); ***4*** animal pretreated with L-arginine (250 mg/kg intraperitoneally) and after 1 h treated with $HgCl_2$ (3 mg/kg intraperitoneally). Data are the mean of 4 experiments ± S.D

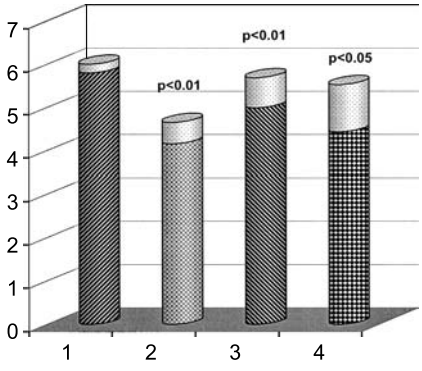

Fig. 3. Activity of liver diamine oxidase (DAO) expressed as units/mg of proteins: ***1*** Control; ***2*** animal treated with $HgCl_2$ (3 mg/kg itraperitoneally); ***3*** animal pretreated with L-arginine (250 mg/kg intraperitoneally); ***4*** animal pretreated with L-arginine (250 mg/kg intraperitoneally) and after 1 h treated with $HgCl_2$ (3 mg/kg intraperitoneally). Data are the mean of 4 experiments ± S.D

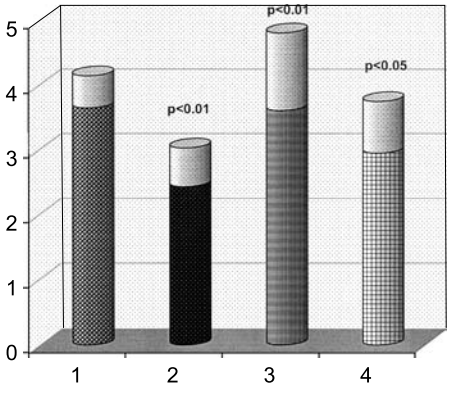

Fig. 4. Activity of liver polyamine oxidase (PAO) expressed as units/mg of proteins: ***1*** Control; ***2*** animal treated with $HgCl_2$ (3 mg/kg kg itraperitoneally); ***3*** animal pretreated with L-arginine (250 mg/kg intraperitoneally); ***4*** animal pretreated with L-arginine (250 mg/kg intraperitoneally) and after 1 h treated with $HgCl_2$ (3 mg/kg intraperitoneally). Data are the mean of 4 experiments ± S.D

increase NOS activity leading to higher production of nitrate/nitrite levels (Fig. 2, group 4).

Polyamine catabolic enzymes, PAO (Fig. 3, group 2) and DAO, (Fig. 4, group 2) were significantly decreased compared to control group (PAO, $p < 0.001$), and DAO, ($p < 0.01$).

Discussion

Arginase is one of six enzymes that play a role in the breakdown and removal of nitrogen from the body by the urea cycle. It is the enzyme that hydrolyses L-arginine to urea and L-ornithine. The main function of arginase in the liver is the detoxification of ammonia. Arginase activity is expressed at other sites, but its exact role in extrahepatic tissues is not well understood. In mammals, two arginase isoforms are expressed: the cytosolic Arginase-1 and the mitochondrial Arginase-2. The isoforms catalyze the same reaction, but are encoded by different genes and differ in their tissue distribution. Arginase-1 is an essential enzyme of the urea cycle and is expressed at high levels in hepatocytes. In mercury-chloride-treated rats the activity of this enzyme was only slightly affected. Unfortunately, the usefulness of arginase as a biomarker in clinical situations is debatable because a variety of factors, including diabetes, liver disease and ingestion of high protein diets, can affect arginase activity (Morris, 1992).

Polyamines are ubiquitous organic cations of low molecular weight. The cellular functions of polyamines, putrescine, spermidine and spermine, is related to the growth and proliferation of mammalian cells. Recent studies indicated that these polycationic compounds might have additional importance in cell functions. Cellular polyamine homeostasis is achieved through balance between biosynthesis, degradation, and uptake. Two amino acids, L-ornithine and L-methionine, are precursor for polyamine synthesis. ODC (EC 4.1.1.17) leads to formation of putrescine from ornithine. In reactions catalyzed by the activities of spermine and spermidine synthase and by adenosine methionine decarboxylase spermidine or spermine can be produced (Jänne et al., 2004).

Polyamines are catabolized via two distinct pathways: the terminal catabolic pathway and the introversion pathway (Pegg and McCann, 1982). The terminal catabolic pathway involves the oxidative deamination of polyamines by copper-containing amine oxidases, generating compounds that cannot be reconverted into polyamines. The interconversion pathway involves DAO (EC 1.4.3.6) which catabolyzes putrescine and PAO (EC 1.5.3.3.), which convert high molecular weight polyamine into low molecular weight amines. Both pathways generate

potentially toxic aldehydes, hydrogen peroxide and ammonia, that can damage proteins, DNA, and lipids (Gaugas and Dewey, 1981; Brunton et al., 1991; Silva, 1996). The presence of an amine oxidase activity is likely essential for the apoptotic effect of polyamines on normal and neoplastic cells (Facchiano et al., 2001).

Polyamine biosynthesis was more investigated than polyamines degradation (Seiler, 2004). Recent findings show that polyamine catabolic enzymes activity could be of importance for the regulation of cell function (Nikolic et al., 2003).

The present study show that decreases of arginase activity directs arginine utilization to NO formation. NO is an important second messenger involved in a variety of physiological processes. NO signal transduction pathways include response to infection, apoptosis, cell proliferation and adhesion, smooth muscle tone, platelet activation, cardiac and skeletal muscle, respiration, neurotransmission and hormone secretion; NO is also a regulator of the activity of many enzymes (Moncada et al., 1991).

A number of regulatory mechanisms exist and interact in metabolic pathways of L-arginine. Arginase modulates NO production in activated macrophages (Chang et al., 1998; Li et al., 2001). N-omega-hydroxy-L-arginine, an intermediary in the production of NO from arginine is a potent inhibitor of arginase activity (Boucher et al., 1994). Agmatine exerts inhibitory effects on both NOS and polyamine pathways, increasing polyamine degradation. Agmatine and NO directly inhibit ODC activity (Szabâo et al., 1994; Schwartz et al., 1997; Satriano et al., 1998; Vargiu et al., 1999). The effects of polyamines are both induction and inhibition of biosynthetic and catabolic enzyme activities that associated with increased and decreased apoptosis. The cells may undergo apoptosis when the polyamine pools are essentially depleted or increased (Gaugas and Dewey, 1981; Schipper et al., 2000; Yu et al., 2003). These controversial results may derive from the recent finding that inhibitors of polyamine oxidation or inhibitors of transglutaminases activity prevented polyamine-induced apoptosis (Facchiano et al., 2001).

Free radical production, enzyme inhibition, binding and dysfunction of thiol-containing proteins are more investigated parameters in mechanisms of mercury chloride toxicity.

The results of our study show increase of NO-synthase activity, which leads to elevation of NO in the liver of intoxicated rats. As is known, NO inhibits cell proliferation by inhibition of ODC, limiting enzyme in polyamine synthesis, in the reaction of NO with Cys360 – nitrosylation reaction (Bauer et al., 2001; Hillary and Pegg, 2003).

Our findings suggest that the increases of plasma liver enzymes, ALT and AST may imply liver damage by mercury chloride. On the other hand the decreases of arginase activity, together with the elevation of nitrate and nitrite levels in liver tissue suggest that arginine is preferentially directed to nitric oxide production. Depletion of ornithine level and possible inhibition of ODC by increased level of NO may result in depletion of tissue levels of polyamine. The observed depressed activity of polyamine catabolic enzymes, PAO and DAO, may be a compensatory response to mercury chloride toxicity in promotion of cell reparation and regeneration. Finally our results indicate that L-arginine and its metabolites have an important role in the regulation of polyamine levels in mercury chloride-induced hepatotoxicity.

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