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Short Communication

LIVER ASPARTATE TRANSAMINASE ISOENZYMES AS BIOMARKERS OF CHRONIC EXPOSURE TO CHROMIUM(VI)

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Exposure to hexavalent chromium compounds is associated with the risk of lung cancer, dermatitis, gastrointestinal ulcers, and other tissue damages. The aim of this study was to compare liver isoenzyme and total serum activities of aspartate aminotransferase (AST) as cytotoxic biomarkers of acute and chronic cytotoxicity of Cr^{VI}. We investigated the extent of cell damage caused by chromium(VI) in acute (2.5 mg kg^{-1}) daily doses administered over five days and chronic (0.25 mg kg^{-1} and 0.5 mg kg^{-1}) daily doses administered over 15 to 60 days by measuring total AST in serum and low molecular weight AST (LMW-AST) and high molecular weight AST (HMW-AST) activities in thirty liver fractions. We also evaluated the kinetic properties and electrophoretic mobility of the LMW- and HMW-AST isoenzymes in liver subcellular fractions. Liver LMW-AST and total serum AST activities significantly decreased after 15 days of exposure ($P<0.05$). With continued treatment, AST activity increased by 15.67 % ($P<0.05$). Interestingly, changes in serum AST activity were similar to changes in the liver LMW-AST isoenzyme. Our results confirmed that total serum AST activity may serve as a reliable tissue biomarker for long-term exposures to Cr^{VI}, but they also suggest that the LMW-AST isoenzyme could be even more sensitive.

KEY WORDS: *biomarker, enzyme activity, hexavalent chromium, HMW-AST, LMW-AST*

Hexavalent chromium (Cr^{VI}) compounds (chromate and dichromate) are used in the production of stainless steel, wood preservation, leather tanning, textile dyes, and conversion coatings. In many occupations, workers handling chromium(VI)-containing products are at the risk of lung cancer, dermatitis, and gastrointestinal ulcers (1, 2). Hexavalent chromium is transported into the cell via non-specific anion transport systems (3, 4), where it converts to trivalent chromium (Cr^{III}), whose highest levels have been reported in the kidney, liver, spleen, and bones (5). Although cytotoxic mechanisms are not exactly understood, several studies have showed that Cr^{VI} induces oxidative stress, DNA damage, single-and double-strand breaks, and affects survival signalling pathways (6-8). This damage and molecular events are believed to be induced by free radicals (HO° and R°) produced during chromium oxidation/reduction (9).

Costa et al. (10) have reported that Cr^{VI} causes mitochondrial dysfunction, since it reduces oxygen consumption in a concentration-dependent manner. Some isoenzymes such as those of aspartate aminotransferase (AST) are found in the cytosol (c-AST) and mitochondria (m-AST) of liver, heart, muscle, pancreas, and kidney cells. Therefore, cellular

leakage of these isoenzymes into the circulation, and signalling responses may indicate tissue damage due to chromium cytotoxicity.

The aim of this study was to compare liver isoenzymes and total serum activities of AST as cytotoxicity biomarkers of acute and chronic exposure to Cr^{VI}.

MATERIALS AND METHODS

Animals and treatment

The study included 55 six-week-old male Wistar rats weighing 200 g to 250 g bred at the Isfahan University of Medical Sciences Central Animal House in standard conditions. The experiment observed the national law on animal protection.

Acute effects were measured in five rats that were receiving intraperitoneal (*i.p.*) doses of 2.5 mg kg^{-1} day^{-1} Cr^{VI} in the form of dichromate salt for five days and compared with the control group of five animals that received saline only.

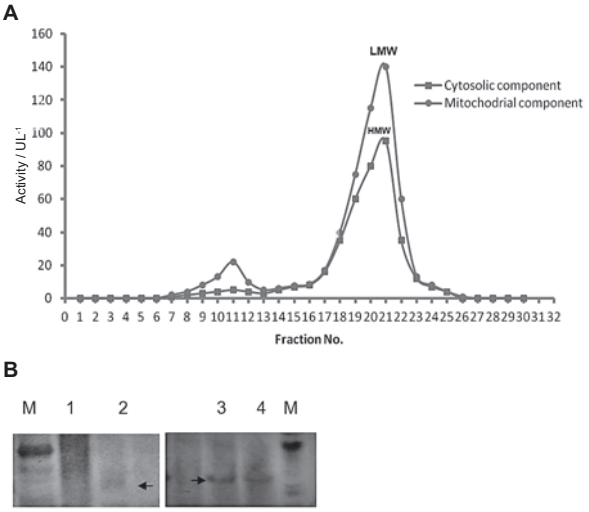

Figure 1 *Subcellular fractionation of rat liver cells.* ***A*** *- cytosolic and mitochondrial components separated from liver homogenate by centrifugation (15000g, 30 min) and loaded on the sephacryl S-300 column. Chromatography of 30 liver fractions showed two separate peaks corresponding to HMW-AST and LMW-AST isoenzymes.* ***B*** *- Pooled HMW-AST and LMW-AST fractions electrophoresed on polyacrylamide gel. 1 - HMW-AST fraction; 2 - HMW-AST fraction after partial purification with $CdCl_2$ (3 mmol L^{-1}); 3 - LMW-AST fraction after partial purification with $CdCl_2$ (3 mmol L^{-1}); 4- LMW-AST fraction; M - marker.*

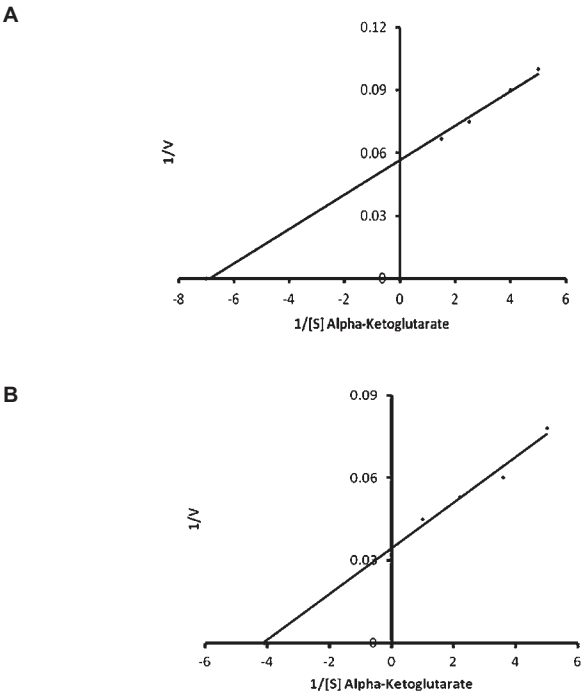

Figure 2 *Alpha-ketoglutarate Km_{app} for HMW-AST (**A**) and LMW-AST (**B**) on Lineweaver-Burk plots*

To evaluate chronic effects, we divided 45 rats in three groups of 15, each consisting of one subgroup receiving 0.25 mg kg^{-1} day^{-1} of Cr^{VI}, one subgroup receiving 0.5 mg kg^{-1} day^{-1}, and one control group receiving saline, but differing in the length of exposure as follows: 15 days, 45 days, and 60 days. The choice of chronic doses was based on an earlier study (11). All experimental and saline doses were injected in daily volumes of 0.2 mL. Animals were killed by decapitation 24 h after the last dose, and blood samples (2 mL to 3 mL) collected in pre-washed plastic tubes. Livers were excised immediately and rinsed of blood with PBS buffer (NaCl, 134 mmol L^{-1}; KCl, 2.7 mmol L^{-1}; Na_2HPO_4, 10 mmol L^{-1}; KH_2PO_4, 2 mmol L^{-1}; pH 7.4). All samples were stored at -20 °C for analysis.

All chemicals (of analytical grade) and materials used were purchased from Sigma Chemical Co. (USA).

Serum and liver AST analysis

Liver tissue (1 g) was homogenised with five volumes of sucrose solution (25 mmol L^{-1}), and the homogenate (0.5 mL) and serum (0.5 mL) loaded on top of the filtration column (1x50 cm) packed with Sephacryl S-300 gel. The column was eluted with Tris-HCl buffer (25 mmol L^{-1}, pH 7.5), and thirty

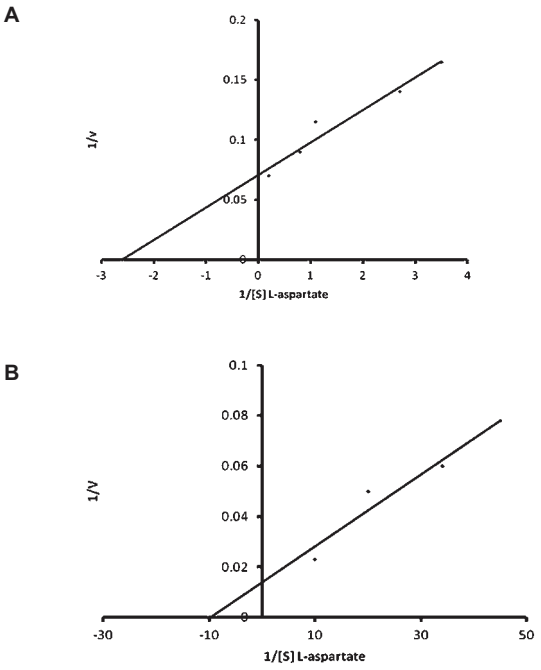

Figure 3 *L-aspartate Km_{app} for HMW-AST (**A**) and LMW-AST (**B**) on Lineweaver-Burk plots.*

fractions (2 mL) prepared to evaluate HMW-AST and LMW-AST isoenzyme activities. The HMW-AST and LMW-AST fractions were pooled in two tubes and additionally purified using the salting out method ($CdCl_2$, final concentration: 3 mmol L^{-1}). After centrifugation (15,000*g* for 30 min), the supernatants were electrophoresed on polyacrylamide gel (21 %; 110 V) and stained with coomassie blue to compare electrophoretic mobility.

The apparent Michaelis-Menton constant **(**Km_{app}**)** of α-ketoglutarate (0.13 mmol L^{-1} to 1 mmol L^{-1}) and L-aspartate (0.025 mmol L^{-1} to 0.1 mmol L^{-1}) substrates were estimated on Lineweaver-Burk plots for both HMW-AST and LMW-AST isoenzymes.

Total serum AST activity (12) was measured with a UV–VIS spectrophotometer (Perkin–Elmer, USA) at 505 nm using the Darman-Kave kit (Isfahan, Iran). In kinetic studies, AST activity (13) was measured at 340 nm in a ping-pong mode for each substrate. Protein concentration of each fraction was measured using Lowry's method (14) and was used to calculate specific activity.

Statistical analysis

Data are expressed as mean±SD. Student's *t*-test was used to identify differences between treated groups and respective controls using Epi Info software 6.04 (CDC, Atlanta, GA, USA). A value of $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

LMW-AST and HMW-AST isoenzymes

Figure 1A shows two activity peaks for HMW-AST and LMW-AST isoenzymes when the homogenates are eluted on the chromatograph column. The pooled fractions for each isoenzyme were further purified with $CdCl_2$ so that LMW-AST and HMW-AST isoenzyme yields were higher than 90 % and the respective purification folds were 18 and 59 times, respectively. Electrophoretic mobility of HMW-AST was greater than that of LMW-AST (Figure 1B). Alpha-ketoglutarate Km_{app} for HMW-AST (0.12 mmol L^{-1}) was lower than that of LMW-AST (0.24 mmol L^{-1}) (Figure 2, A and B), whereas the opposite was true for L-aspartate Km_{app} (0.37 mmol L^{-1} *vs.* 0.1 mmol L^{-1} for HMW-AST and LMW-AST fractions, respectively (Figure 3, A and B). Our results have shown that HMW- and LMW-AST isoenzymes can be separated on the gel filtration column and that their biochemical characteristics correspond to cytosolic and mitochondrial isoenzymes.

Liver LMW-AST isoenzyme activity dropped in rats treated for 15 days and increased in the 45-day and 60-day groups (Figure 4A). We believe that these differences between the groups are related to lower mitochondrial oxygen consumption, attenuated gene expression in short-term exposure (15, 16), and increased mitochondrial/microsomal lipid peroxidation, cellular induction, and failure in membrane integrity in longer exposures (17).

Similar to LMW-AST, liver HMW-AST activity dropped in the 15-day-treatment group, but it also dropped in the 60-day group, whereas in the 45-day group it increased (Figure 4B). As with LMW-AST, changes in liver HMW-AST may reflect lower gene expression in short-term exposure and then rise in the 45-day group as a consequence of suppression of chromium-induced oxidative stress by endogenous vitamin C biosynthesis, as reported by Mohan et al. (18) and Hemmati et al. (19). Lower HMW-AST activity in long-term (60-day) exposure may reflect oxidative stress and the development of apoptosis (20) in soft tissues (21-23).

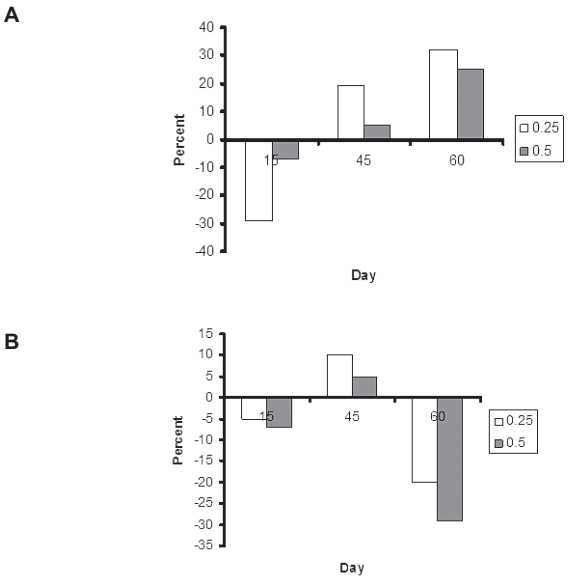

Figure 4 *Changes in liver HMW-AST and LMW-AST activities. The activities of pooled LMW-AST (A) and HMW-AST (B) fractions measured after 15, 45, and 60-day treatment with 0.25 mg kg^{-1} or 0.5 mg kg^{-1} of Cr^{VI} compared to control.*

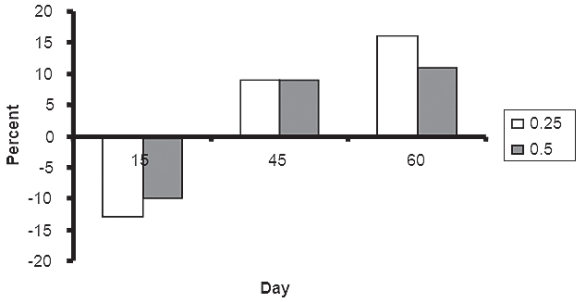

Figure 5 *Changes in total serum AST activity in rats receiving Cr^{VI} doses of 0.25 mg kg^{-1} and 0.5 mg kg^{-1} over 15, 45, and 60 days compared to control.*

Total serum AST

Acute Cr^{VI} dose (2.5 mg kg^{-1}) lowered serum AST activity significantly (P<0.005) by 20.7 % (Table 1). Similarly, Krumschnabel and Nawaz (24) showed that acute exposure to Cr^{VI} may significantly decrease hepatocyte viability. Other studies (4, 25) also showed that Cr^{VI} converts to Cr^{III} and inhibits the enzyme biosynthesis.

In the group treated for 15 days, total serum AST was still 12.82 % lower than control's (P<0.01) and then started to rise gradually by the end of the experiment, reaching 15.67 % increase (P<0.03) in the 60-day group (Table 1, Figure 5). Clearly, continued exposure to Cr^{VI} increases the release of AST into the bloodstream, probably because of cell deterioration and hypoxic response, as described elsewhere (26).

CONCLUSION

Our results have shown that, in contrast to liver HMW-AST, liver LMW-AST isoenzyme and total serum AST activities increase in long-term exposure to Cr^{VI}. In addition, a comparison between LMW-AST and total serum AST showed that LMW-AST is more sensitive biomarker of Cr^{VI} toxicity. These findings are yet to be verified by future non-invasive studies of human serum AST isoenzymes.

Table 1 *Total serum AST activity*

Treatment	Days of treatment	Group	Cr^{VI} dose / mg kg^{-1}	AST activity / IU L^{-1} Mean±SD	Change / %	Difference from control
Acute	5	Control	Saline	114.87±3.32	-	-
	5	Treated	2.5	91.08±7.51	-20.7	<0.005
Chronic	15	Control	Saline	117.31±4.70	-	-
	15	Treated	0.25	101.98±2.39	-12.82	<0.01
	15	Treated	0.5	105.12±2.44	-10.08	<0.02
	45	Control	Saline	106.21±6.12	-	-
	45	Treated	0.25	115.41±6.44	+8.87	NS
	45	Treated	0.5	115.75±5.76	+9.21	NS
	60	Control	Saline	108.21±5.12	-	-
	60	Treated	0.25	125.17±9.62	+15.67	<0.03
	60	Treated	0.5	120.43±5.21	+11.29	<0.03

*NS - Not-significant (*P*>0.05).*

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Sažetak

JETRENI IZOENZIMI ASPARTAT TRANSAMINAZE KAO BIOPOKAZATELJI KRONIČNE IZLOŽENOSTI HEKSAVALENTNOM KROMU

Izloženost spojevima s heksavalentnim kromom (Cr^{VI}) povezana je s povećanim rizikom od raka pluća, dermatitisa, vrijeda u probavnom traktu i ostalih oštećenja tkiva. Cilj ovog istraživanja bio je na životinjskom modelu usporediti aktivnosti aspartat aminotransferaze (AST) u serumu i njezinih jetrenih izoenzima kao biopokazatelja akutne i kronične citotoksičnosti Cr^{VI} u štakora. S tom smo svrhom pet dana intraperitonealno izlagali Wistar štakore akutnoj dozi Cr^{VI} od 2,5 mg kg^{-1} na dan te petnaest, četrdeset pet i šezdeset dana kroničnim dozama od 0,25 mg kg^{-1} odnosno 0,5 mg kg^{-1} na dan, te izmjerili aktivnost ukupnog AST u serumu i aktivnosti izoenzima AST-a male (LMW-AST) odnosno velike molekulske mase (HMW-AST) u 30 jetrenih frakcija. Također smo ocijenili kinetička svojstva i elektroforetsku mobilnost LMW-AST-a i HMW-AST-a unutar jetrenih stanica. Aktivnosti jetrenog LMW izoenzima AST-a i ukupnog AST-a u serumu značajno su se smanjile nakon 15 dana izloženosti u odnosu na kontrolnu skupinu (P<0,05). Tijekom daljnje izloženosti aktivnost serumskog AST-a povećala se 15,67 % (P<0,05). Zanimljivo je primijetiti da je ponašanje serumskog AST-a bilo slično onom jetrenog LMW izoenzima, što upućuje na pouzdanost obaju biopokazatelja pri dugotrajnoj izloženosti kromu, ali i na bolju osjetljivost LMW-AST izoenzima.

KLJUČNE RIJEČI: *biopokazatelj, enzimska aktivnost, heksavalentni krom, HMW-AST, LMW-AST*

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