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ANIMAL SCIENCE

Effect of arsenic exposure on metabolic activities involving adaptive responses in liver of fresh water fishes (*Channa punctata*)

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Abstract

As a major organ, liver plays the critical role in metabolic regulation, however, the metabolic activities are impaired by both environmental and chemical stimuli. *Channa punctata* variety of fishes was exposed to different concentration of sodium arsenate (Na_2HAsO_4) as the fishes are energetic and survive in the critical environment. Both 1 mM and 10 mM Na_2HAsO_4 enhanced protein content in liver for 1 h and 2 h, however, the effect was higher for 10 mM concentration. The fishes exposed to 100 mM Na_2HAsO_4 also causes increase in protein and the effect was lower than the previous doses. The inorganic phosphate (Pi) level was increased significantly whenever the fishes were exposed to Na_2HAsO_4 and found to be higher for 1 mM dose. The increased Pi release was also observed in response to higher dose, however, lower than the previous doses. Similar stimulatory effects on alkaline phosphatase activity were observed in liver exposed to the above concentrations and were found to be maximal for 1 mM dose. The results appear to indicate that the adaptive response involving the higher synthesis of molecules induced by arsenic is responsible for survive of the species and will give a new insight for the regulation of metabolic activities in liver and might be an index for characterization of the pathology of fishes.

Key words: Arsenic exposure, *Channa*, Liver, Metabolic regulation, Adaptive response

Introduction

Arsenic is an important and ubiquitous environmental toxicant and the risk of arsenic poisoning in human is a public health issue worldwide (Ahmad et al., 1997; Tchounwou et al., 2003). In addition, arsenic is classified as a human carcinogen based on several epidemiological studies showing an association of arsenic exposure with cancers in lung, bladder, kidney and liver (Hughes, 2002). Despite these findings and the fact that arsenic is the most extensively studied of the meals and metalloids in drinking water, the molecular mechanisms of arsenic toxicity is poorly understood.

The liver is a major target organ of arsenic toxicity in both mice (Waalkes et al., 2003; Wang et al., 2002) and human (Chen et al., 1986; Chen et al., 1992). Arsenic-induced liver injury in humans is a common phenomenon, typically manifesting initially as degenerative lesions with jaundice, progressing to noncirrhotic portal hypertension, fibrosis, cirrhosis and neoplasia such as hepacellular carcinoma (Centeno et al., 2002; Lu et al., 2001). Although many arsenic-induced differentially expressed genes have been identified, there are no data on *in vivo* kinetics on the adaptive response of a specific targeted organ, such as liver, the experimental results should aid further understanding of arsenic mechanisms of toxicity resulting in pathology.

Channa punctatus is generally found in fresh water of haor, bil, river in Bangladesh. They are much energetic and survive in the critical circumstances for long time, for example, water deprivation. They are used as a source of protein in the diet for human being. It is assumed that the higher energy content of this fish is caused by the increased activity of the sympathetic nerves. During environmental adverse condition, liver might be involved critically on its regulation of metabolites to survive in the atmosphere. However, to survive in the atmosphere caused by toxic arsenic, the critical role of liver of these species on adaptive response involving the regulation of metabolic processes is not understood.

As a metabolic organ, liver plays a major role in biotransformation of foreign toxic substances. Therefore, the organ may also serve as a regulatory

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area to the sensitivity of toxic substances. Liver glycogenolysis is a metabolic process by which the energy is released for doing mechanical work and there by the species may use the energy to survive. Besides glycogenolysis, other metabolic functions are also involved in the survival process, although the mechanism of the survival process and the toxic effect of arsenic causing metabolic alterations through expression of pathological syndromes are not known. Prolonged exposure of arsenic has detrimental effects in tissues. It may impair the glycolysis as well as the oxidative processes (Tchounwou et al., 2003) and causes different types of pathogenic syndromes in rodents, fishes and other organisms. Exposure of higher concentration of arsenic in water may also cause severe effects in fish and might be involved in producing cancer or other cellular effects. However, the mechanism underlying the effects of acute arsenic exposure on the regulation of oxidative and glycolytic processes in liver is not well understood. The accumulation of arsenic to the living organism is mediated directly or from other species. Moreover, fish have long been used as sentinels for biomonitoring of aquatic environmental pollutants and are good indicators of arsenic toxicity (Tisler et al., 2002). The regulation of metabolic activities in liver in response to the toxic arsenic is an important aspect in fish and to clarify the role of arsenic in liver metabolic functions responsible for survive of the species of fishes in the environment and characterization of pathogenesis of this species since protein, Pi and alkaline phosphatase are the metabolic index for characterization of liver pathogenesis, the current protocol was designed.

Materials and Methods

Fishes

Channa punctatus weighing 50 g to 60 g were used. They were maintained in normal water with ambient temperature (25.0 ± 1.0°C). In the day of experiment, arsenic exposure was given to the different groups of fishes in plastic pot. After the treatment, fishes were quickly decapitated and liver was sampled carefully and taken weight by digital balance (Chyo, JL-180, China) and kept at –20^{o}C. Control fishes were similarly used for sampling of tissues except giving arsenic exposure.

Arsenic treatment

To examine the role of arsenic on the regulation of metabolic activities in liver, groups of fishes were exposed with different concentrations of arsenic compound (1 mM, 10 mM and 100 mM Na_2HAsO_4. $7H_2O$, BDH Chemical Ltd.). Five fishes in each group were kept in a transparent plastic pot (size 10"X10"X5"). The fishes were bought from the nearby market and maintained in normal water in the laboratory for a while without any food. During the experiment, no foods were given in the pot and both 1 mM and 10 mM arsenic exposure were done for 1 h and 2 h, however, the other groups of fishes were treated with only 100 mM of arsenic compound (Na_2HAsO_4) for 1 h in ambient temperature. The control fishes in separate pot were used with normal water only.

Assay of tissue metabolite

Liver was homogenized with pre-cooled water and was centrifuged at 8000 rpm for 10 min. The supernatant was used as crude extract for assay of protein, inorganic phosphate and alkaline phosphatase (ALKP) activity. Protein was determined by Lowry et al. (1951) method by using 50 µl crude extract. Inorganic phosphate and alkaline phosphatase activity were determined as described by Ramnik (1999) by using 100~200 µl extract. For Pi estimation, 200 µL tissue extract was diluted to 5 mL with water and was mixed vigorously with 5 mL of 5% TCA (Trichloroacetic acid) and centrifuged at 6000 rpm for 10 min. 5 mL supernatant was transferred to another tube and kept on ice. 1 mL molybdate reagent (10 g of ammonium molybdate in 100 mL water was taken and 100 mL of 5N H_2SO_4 was added to prepare 200 mL solutions) was added and mixed. The solution was mixed with 0.4 mL aminonaptholsulphonic acid reagent. 3.6 mL water was added and after mixing, the tube was kept standing for 10 min for the complete development of color. For blank, 5 mL of 5% TCA and 5 mL water were mixed only. Absorbance was taken at 690 nm against the blank. The Pi in tissue extract was calculated using standard KH_2PO_4 solution.

For assay of alkaline phosphatase activity, 0.25 mL of PNPP (p-nitrophenyl phosphate (1.2 mg/mL in glycine-NaOH buffer, pH 10.0) was added to 0.5 mL glycine-NaOH buffer (pH 10.0) and incubated for 5 min at 37°C. 100~200 µL of tissue extract was taken to the solution and for blank, same volume of buffer was used in place of tissue extract and incubated for 30 min. After incubation, it was made up to 4 mL with 0.1N NaOH solution and absorbance was taken at 410 nm. The amount of PNP (p-nitrophenol) produced after hydrolysis of PNPP) by the enzyme was measured from the standard PNP solution (500 µmolL^{-1} in buffer, pH 10.0). The enzyme activity is expressed as µmol of PNP $min^{-1}g^{-1}$ of tissue.

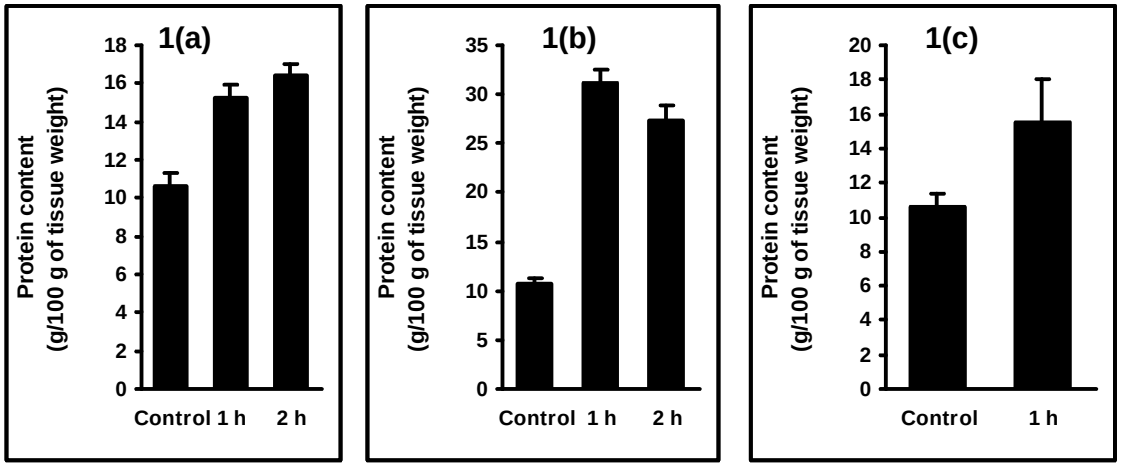

Figure 1. Changes of protein content in liver of fishes exposed to 1 mM (a), 10 mM (b) and 100 mM (c) Na_2HAsO_4. The fishes were kept for 1 h and 2 h in arsenic solution, however, for 100 mM concentration; they were kept for 1 h. The data are average ± SEM for 4~5 fishes in each group.

Statistical analysis

Results of the experiments were expressed as mean and standard error of different groups. The differences between the mean values were evaluated by ANOVA followed by pared t-test using SPSS software.

Results

Effects of 1 mM, 10 mM and 100 mM Na_2HAsO_4 on protein content in liver

As shown in Figure 1 (a), the average protein in liver in response to 1 mM arsenic for 1 h and 2 h were 15.24 ± 0.73 g and 16.47 ± 0.53 g respectively whereas for control fishes, the amount of protein was 10.63 ± 0.72 g/100 g of tissue weight. A significant 43.36% (P<0.001) and 54.93% (P<0.05) increased protein was observed after 1 h and 2 h respectively when compared with control liver. Groups of fishes were exposed to 10 mM arsenic solution and the amounts of protein for 1 h and 2 h were 31.20 ± 1.22 g and 27.37 ± 1.53 g/ 100 g of liver respectively. The results indicated that 193.50% (P<0.001) and 157.47% (P<0.001) increased protein had been found respectively after 1 h and 2 h in response to 10 mM Na_2HAsO_4 compared to the control fishes, shown in Figure 1 (b). The results also appear to indicate that 10 mM arsenic causes higher protein content than 1 mM concentration.

The amount of protein in liver of other groups of fishes in response to higher arsenic level (100 mM Na_2HAsO_4) for 1 h was 15.57 ± 2.42 g and for control, the value was 10.63 ± 0.72 g /100 g of tissue weight, shown in Figure 1 (c). The results indicate that 100 mM Na_2HAsO_4 causes also increased protein by 46.47% (P<0.1), however, lower than the previous doses.

Effects of 1 mM, 10 mM and 100 mM Na_2HAsO_4 on inorganic phosphate (Pi) level in liver

To examine the role of arsenic on Pi release, fishes were exposed to 1 mM Na_2HAsO_4 for 1 h and 2 h and the amount of Pi releases were 12.67 ± 0.79 mg and 12.76 ± 1.33 mg /100 g of tissue weight while for the control fishes, the value was 2.68 ± 0.19 mg/100 g of tissue. The Pi values in livers of arsenic-treated fishes were increased significantly by 372.76% (P<0.001) and 376.11% (P<0.001) respectively for 1 h and 2 h than in livers of control fishes, Figure 2 (a). In separate examinations, groups of fishes exposed with 10 mM Na_2HAsO_4 had 9.26 ± 0.78 mg and 3.42 ± 0.33 mg Pi after 1 h and 2 h respectively. The Pi content in liver was increased similarly significantly by 245.52% (P<0.001) and 27.61% (P<0.05) respectively when compared to the control as demonstrated by Figure 2 (b). The results demonstrated that arsenic causes stimulatory effect on Pi release, however, the effects were more pronounced when the fishes were exposed to 1 mM arsenic compound than 10 mM concentration.

As shown in Figure 2 (c), the increased Pi content in liver in response to 100 mM Na_2HAsO_4 for 1 h was 3.59 ± 0.59 mg while for control fish, the value was 2.68 ± 0.19 mg/100g of tissue. Arsenic causes increased Pi by 33.95% (P<0.001) and the effect was lower than the previous doses.

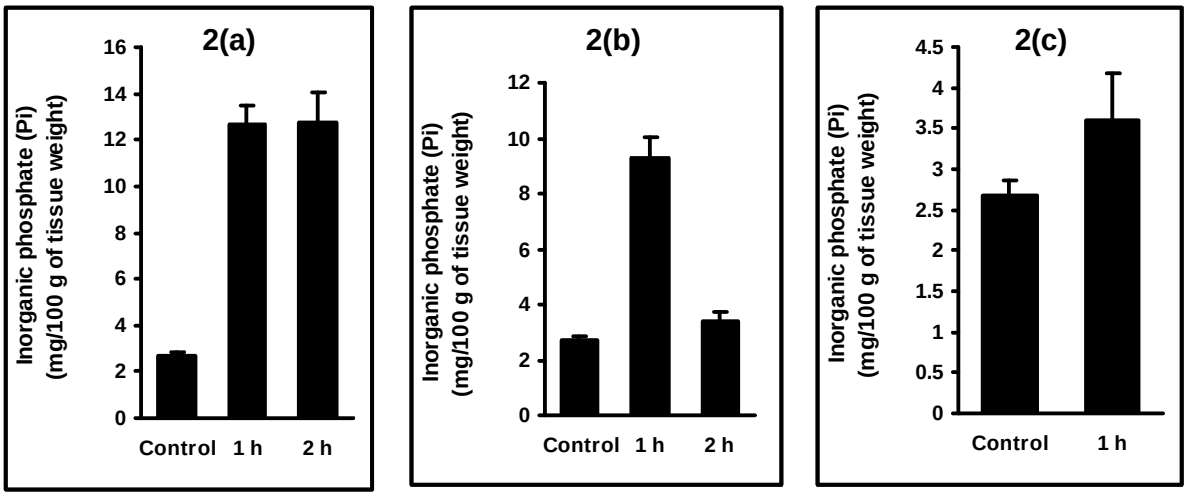

Figure 2 Changes of inorganic phosphate (Pi) in liver of fishes exposed to 1 mM (a), 10 mM (b) and 100 mM (c) Na_2HAsO_4. The fishes were exposed to arsenic for 1 h and 2 h, while for 100 mM concentration; they were kept for 1 h. The data are average ± SEM for 4~5 fishes in each group, however, 4 fishes were used for 100 mM concentration only.

Therefore, it is assumed that the lower arsenic level might be effective for producing higher Pi release from the liver. The results suggest that the increased Pi might be due to the higher activity of some enzymes responsible for the degradation of the cellular organic compounds and could be considered as the survival factor for this species in critical environment.

Effects of 1 mM, 10 mM and 100 mM Na_2HAsO_4 on alkaline phosphatase (ALKP) activity in liver

Liver ALKP is sensitive to toxic response and releases Pi from PNPP. To examine the role of arsenic on ALKP activity in liver, groups of fishes were treated with 1 mM and 10 mM Na_2HAsO_4 for 1 h and 2 h and the control livers of fishes kept in ambient temperature were also examined. The ALKP activities in response to 1 mM Na_2HAsO_4 for 1 h and 2 h were 2.68 ± 0.21 μmol and 2.70 ± 0.23 μmol respectively whereas for control fishes, the activity was 0.35 ± 0.08 μmol/min/g of tissue. The results demonstrated that ALKP activities had been significantly enhanced and stimulated (665.71%, $P<0.001$ and 671.42%, $P<0.001$ respectively) by arsenic compared to control, however, the activity was appeared to be higher for 2 h exposure as shown in Figure 3 (a). The ALKP activities in livers of other groups of fishes in response to 10 mM Na_2HAsO_4 were 1.28 ± 0.04 μmol and 1.00 ± 0.05 μmol respectively. A significant increased response on ALKP activity was observed for fishes exposed to arsenic (265.71%, $P<0.05$ for 1 h and 185.71%, $P<0.01$ for 2 h), Figure 3 (b), however, the effects were lower than 1 mM concentration, Figure 3 (a), Figure 3 (b).

As shown in Figure 3 (c), the ALKP activity in presence of 100 mM Na_2HAsO_4 for 1 h was 0.93 ± 0.12 μmol while for control fish, the value was 0.35 ± 0.08 μmol/min/g of tissue. Arsenic causes increased activity ($P<0.05$) by 165.71%, however, the effect was lower than the previous doses. The results suggest that the increased ALKP in liver might be due to the toxic effect of arsenic which produces the toxic environment where they survive and could be considered as the survival factor as well as index for characterization of the pathology of liver for this species.

Discussion

The increased protein caused by different concentrations of arsenic (1, 10 and 100 mM Na_2HAsO_4) exposure has been found in liver of fishes in our study. Arsenic produced the toxic environment in water where the fishes want to survive. Therefore, it is reasonable that an adaptive response by the species was created and some stress proteins were synthesized. The liver is the organ where most of the biotransformation of inorganic arsenic takes place (Del Razo et al., 2001). Up regulation of several genes in arsenic-induced adaptive response has been observed (Verma et al., 2002; Chelbi-alix et al., 2003). Their findings suggest that arsenic may induce the synthesis of molecules responsible for the survival process. The liver is a major target organ of arsenic toxicity.

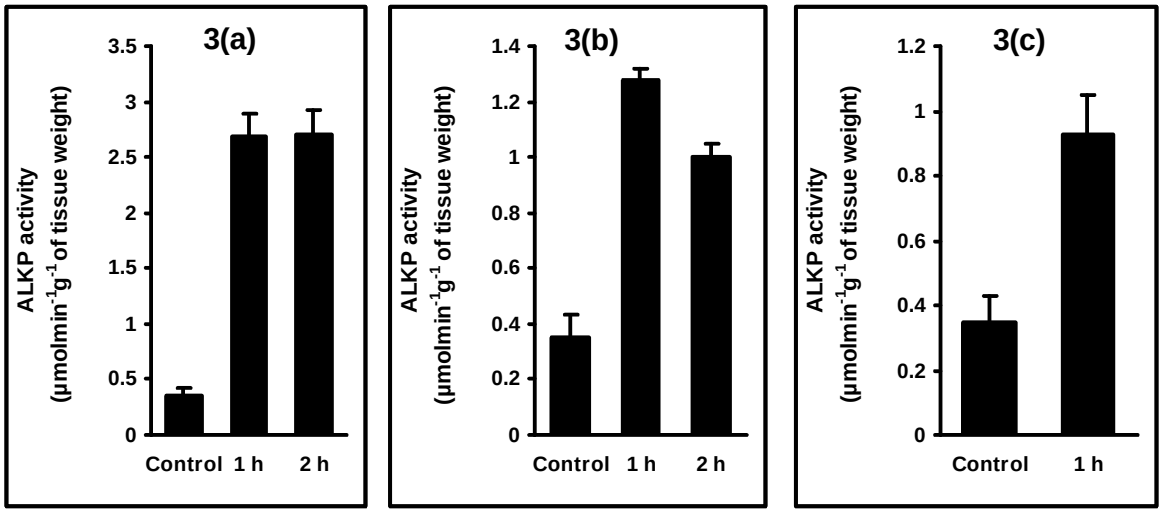

Figure 3 Changes of alkaline phosphatase (ALKP) activity in liver of fishes exposed to 1 mM (a), 10 mM (b) and 100 mM (c) Na_2HAsO_4. The groups of fishes were exposed to arsenic solution for 1 h and 2 h, while for 100 mM concentration; they were kept for 1 h. The data are average ± SEM for 4~5 fishes in each group, however, 4 fishes were used for 100 mM concentration only.

Arsenic-induced liver injury in humans is a common phenomenon, typically manifesting initially as degenerative lesions with jaundice, progressing to noncirrhotic portal hypertension, fibrosis, cirrhosis, and neoplasia such as hepatocellular carcinoma (Mazumder et al., 1998; Lu et al., 2001; Centeno et al., 2002). Prolonged exposure of higher concentration of arsenic has been involved in liver injury and damage (Taylor et al., 1989; Chiou et al., 1995). Liver metabolism is a known potential target for the toxic action of chemicals (Hinton et al., 2001).

Severe Pi release was observed in response to 1 mM Na_2HasO_4, however, both 10 mM and 100 mM Na_2HasO_4 also induced Pi release significantly in liver. It is assumed that 1 mM arsenic effectively and maximally enhanced Pi content. Several molecules might be involved for causing Pi release. The phosphorylase enzymes and phosphatases release Pi whenever they are activated. In the biological system, phosphorylase and the phosphatase are dominantly expressed and are involved in releasing inorganic phosphate. Because of the importance of Pi in biological systems and in medical analysis, a variety of assays have been developed for this ion. Even ALKP also causes the release of Pi upon activation (Nixon et al., 1998; Christenson, 1997). We found that ALKP was significantly increased in response to different concentration of arsenic. Further experiments are needed for clarification of the mechanism of enhancing the Pi release in liver of fishes treated with arsenic. Allen et al. (2004) found that arsenic impairs the sympathetic nerve activity; therefore, the toxic arsenic might be involved in denervation of the similar nerves. Therefore, sympathetic denervations induced by arsenic may play the role for changing the amount of Pi through phosphorylation-dephosphorylation reactions since the tissue is enriched with the similar nerves. It has been demonstrated that inactivation of the protein PP2A is mediated by cold acclimation recognized to be the major sympathetic stimulus (Leduc, 1961) resulting enhancement of phosphorylation process (Antonio et al., 1998). Therefore, the increased Pi in response to arsenic in liver might be due to the higher dephosphorylation process. Because Ca^{2+} enhances the inactivation of protein phosphatase 2A (PP2A) (Antonio et al., 1998), it could be a negative modulator for the higher Pi release. As a peripheral tissue, liver may participate in the survival process during critical circumstances through protein phosphorylation-dephosphorylation mechanisms.

Alkaline phosphatase is predominantly found in liver and is an index of the characterization for liver pathogenesis. The major isozymes of ALKP include those of liver, bone and kidney and those of the intestine and the placenta; the isozymes of these two groups are each encoded by a separate gene (Toe et al., 1989; Weiss et al., 1989). Although this glycoprotein is widely distributed in vertebrate

tissues, its physiological function is as yet fully understood. The released Pi might be influenced in response to low dose of arsenic (1 mM) because ALKP activity is severely augmented than other doses, however, the activity was appeared to be reduced in higher concentrations. The results would indicate that metabolic function involving higher ALKP activity in liver is sensitive to this dose. Recent investigation revealed that arsenic exposure stimulated ALKP in liver (Sharma et al., 2007) and is there by supported the present findings. The higher effect of arsenic in liver demonstrates that increased Pi release would be a survival factor during energy deficiency.

Collectively, as a peripheral tissue, liver is metabolically important for energy consumption and energy expenditure. Environmental toxic arsenic is a major stimulus exerting its effect on metabolic changes. As a major metabolic site, liver plays the critical role in the biotransformation of foreign toxic substances. The diverse metabolite regulation is an index concerning survival of these species as well as characterization of liver pathogenesis and is a biological process; however, arsenic probably takes part in modulation of the metabolic process.

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