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The dynamic effects of different inorganic arsenic species in crucian carp (*Carassius auratus*) liver during chronic dietborne exposure: Bioaccumulation, biotransformation and oxidative stress

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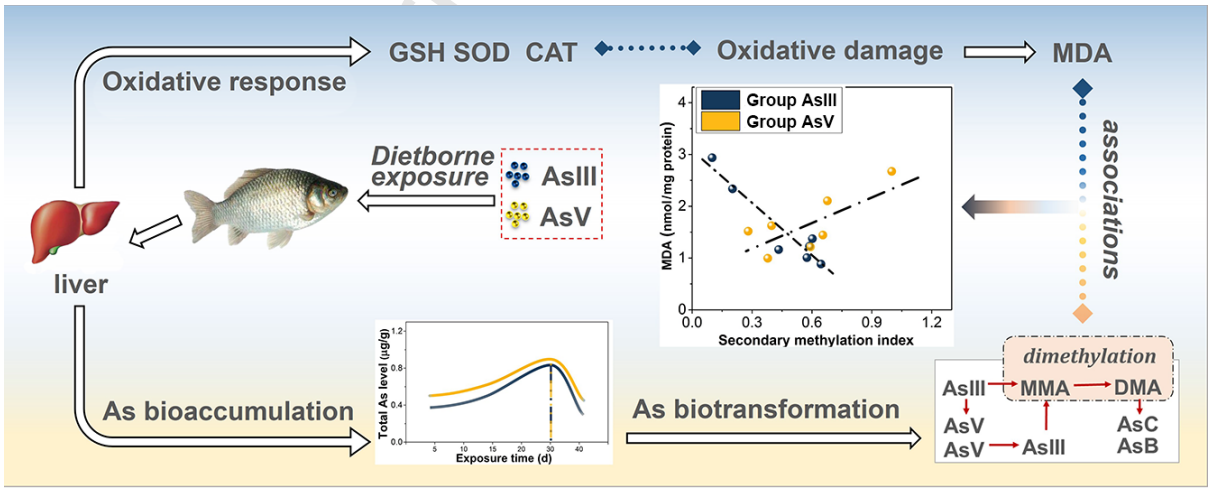

Graphical Abstract

Abstract: Inorganic arsenic (iAs) is highly toxic to aquatic species, but the chronic effect of iAs on fish following dietborne exposure is still unclear. In this study, freshwater fish crucian carp (*Carassius auratus*) was exposed to iAs [arsenite (AsIII) and arsenate (AsV)] for 40 days through dietary exposure. The bioaccumulation and biotransformation of arsenic in the main metabolic organ, liver, were measured. The oxidative stress responses to iAs exposure in liver were analyzed to be linked to arsenic biotransformation, especially methylation. In both AsIII and AsV groups, the total As contents gradually increased during the exposure and then fleetly decreased at the end of exposure (40 d). Arsenobetaine was found to be the predominated As species (34- 66%) and the fraction remained on an increasing trend, while the inorganic As percentages decreased 84-91% during the 40-day exposure, suggesting that the capability of As biotransformation increased to acclimate iAs during chronic dietborne exposure. Both the activities of the enzymatic antioxidants (superoxide dismutase and catalase) and the level of the nonenzymatic antioxidant (glutathione) increased initially and then decreased, thus lowering the malondialdehyde levels and displaying a typical antioxidant defense mechanism. The opposite correlations were observed between arsenic secondary methylation index and the malondialdehyde level in different iAs treatment. This indicated that the As dimethylation played an significant role toward oxidative damage; the toxic action of As dimethylation was dependent upon the parent iAs species at the initial stage of exposure. Therefore, the effectiveness of the detoxification relied on both the biomethylation rate of As and the anti-oxidation ability based on nonenzymatic antioxidant and enzymatic antioxidant.

Keywords: Arsenic, Bioaccumulation, Biotransformation, Oxidative stress, Liver, Freshwater

fish

1. Introduction

Arsenic (As) is ranked the first among the Priority List of Hazardous Substances (https://www.atsdr.cdc.gov/spl/index.html) and is widely distributed in the freshwater sediment as a result of anthropogenic and natural processes (Gorny et al., 2015). The surface sediment can be passively ingested by fish, especially the demersal species. It has been found that the As concentration in demersal fish was positively associated with the As concentration in surface sediment, rather than the concentration of soluble As in water, suggesting that dietborne exposure would be even more influential than waterborne exposure for As accumulation in demersal fish (Hong et al., 2018; Zhang et al., 2018). Assessing risks from As exposure for demersal fish will be incomplete without consideration of the dietborne exposure in cases of As contamination in sediment. However, few data pertaining to the metabolic pathway and toxicological effects of iAs via dietary route for demersal freshwater fish are available.

Inorganic As (iAs), including arsenite (AsIII) and arsenate (AsV), are the primary As species in sediment (Cullen and Reimer, 1989). Following uptake into aquatic organisms, the reduction of AsV to AsIII may occur. The AsIII can be further methylated to monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsenoxide (TMAO), and transformed to more complicated organic species such as arsenocholine (AsC), arsenobetaine (AsB) and various arsenosugars. AsB is one of the less reactive and toxic As

species (Zhang et al., 2016b). In marine fish, 80% of accumulated iAs could be transformed to AsB after dietborne exposure (Zhang et al., 2016a). Although less studied and often performed via waterborne way, the reported As species in freshwater fish is more variable than, and differs substantially from, that in marine fish. AsB has often been reported to be the predominant As species in freshwater fish, but also is often relatively minor, with MMA, DMA, and/or trimethylarsenate being more important (Bears et al., 2006; Cui et al., 2020; Suhendrayatna et al., 2002a; Suhendrayatna et al., 2002b). In addition, these researches ignored the dynamic variation of As species during exposure, and the changes of methylation and toxic As species (AsIII and AsV) in freshwater fish could not be reflected based on the change of the total As level. Therefore, it is necessary to study the dynamic variation of As bioaccumulation and biotransformation during chronic iAs dietborne exposure.

Chronic As exposure could cause a variety of molecular events, including oxidative stress, metabolism disorder, and carcinogenesis, and the dynamic variations in these events require more attention to clarify the chronic toxicity of As (Hughes, 2002). However, most previous researches have focused on the acute As toxicity in fish, less is known about the dynamic effects based on chronic exposure. Enhancement of reactive oxygen species (ROS) levels is a major pathway of As exposure induced toxicity. The excessive ROS may damage major cell macromolecules including DNA, proteins, and lipids, and induce oxidative stress (Altikat et al., 2015; Kumar and Banerjee, 2014). Bagnyukova et al. (2007) found that waterborne exposure of goldfish to AsIII (100-200 μmol/L) caused regulation of the antioxidant enzyme activity and the level of glutathione pool. Chen et al. (2019) reported that iAs waterborne exposure had no significant effect on As bioaccumulation in marine medaka *Oryzias*

melastigma, while significant peroxidation damage was found at the initial stage. Although less studied, continuous exposure to sublethal concentration of As for fish by waterborne route lead to variation in antioxidant biomarkers (Bhattacharya and Bhattacharya, 2007). However, the dynamic effects of dietary exposure to sublethal level of iAs remain ambivalent.

The relationship between iAs toxicity and biotransformation is complicated and remains unclear. AsB is a benign As species and proposed to be the final iAs metabolite in the biotransformation pathway in fish. Therefore, the methylation processes of iAs have long been considered as detoxification mechanisms (Zhang et al., 2016b). However, recent experiments in mammals pointed out the fact that the methylation process could generate trivalent intermediates, MMAIII and DMAIII, which are more active than the precursor iAs in terms of genotoxicity, cytotoxicity, and enzyme inhibition (Dopp et al., 2010). The biotransformation could be a dual course involving both detoxification and activation of iAs. However, further study outcome is needed to define the role of iAs metabolism, that is, whether it is an activation or a detoxification pathway.

We performed an *in vivo* experiment to investigate the dynamic effect of chronic iAs dietborne exposure on bioaccumulation, biotransformation and oxidative stress in liver of crucian carp (*Carassius auratus*). The crucian carp is a freshwater demersal species and commonly used as a model for biotransformation and toxicity studies (Zhang et al., 2004). We chose to concentrate on the liver because it is a significant organ of As accumulation and biotransformation and the proposed target for sublethal As toxicity in fish (Licata et al., 2005). We hypothesized that the rate of As methylation could be important in determining whether

this course is a detoxification mechanism of freshwater fish.

2. Materials and methods

2.1. *Fish and sample collection*

A total of 210 fish (mixed sex, obtained from a fish farm at Changsha, China) with the average length of 14.9 cm were used. The fish were equally divided into AsIII group, AsV group, and control group. In each group, the fish were further distributed into 7 aquaria with each containing 5.0 L dechlorinated municipal water (10 fish per aquarium). Fish were maintained at a water temperature of 24 °C under a light:dark cycle of 12:12 h. Since crucian carp is a demersal species, acclimatization (for 14 days) was required to train them to feed the floating puffed pellet diet (from Hui Ze Biological Technology Co. Ltd, Hu Bei, China) used in the study prior to the exposure experiment. Diet was offered three times a day at about 1% of fish body weight.

Samples of fish liver were collected from a 40-day indoor feeding study. Fish in the control group were fed with basal diet without supplement of As. The AsIII- and AsV- group was fed with AsIII- and AsV- supplemented diet, respectively. The diets were prepared by soaking the basal diet with arsenite ($NaAsO_2$, O2SI, USA) and arsenate ($Na_2HAsO_4 \cdot 7H_2O$, O2SI, USA) solution, respectively, to achieve an environmentally relevant concentration of 50 μg/g (measured as As) (Chai et al., 2017). To prevent interference of waterborne As exposure, the uneaten food was immediately removed from water, and the water was changed every 24 h. On days 0, 5, 10, 15, 20, 30, and 40, seven fish sampled in each group were weighted and euthanized. Then the individual liver was collected immediately and stored in -80 °C for further analyses.

Fish experiments were conducted as prescribed by the guidelines of the animal ethical committee for animal experimentation in China. All efforts were made to minimize fish suffering and the numbers of fish utilized.

2.2. *Determination of total arsenic content in fish liver*

The total As contents in fish livers were measured following previously validated procedures (Jia et al., 2018). About 0.15 g of freeze-dried samples (freeze-drier, Lab-1A-50E, Beijing Boyikang Experimental Instrument Co., China) were weighted and digested with a mixture of 10 mL HNO_3 (70 % wt, ⩾ 99.999 % metal basis, Aladdin Reagent Co. Ltd., China) and 2 mL H_2O_2 (30 % wt, guaranteed reagent grade, Aladdin Reagent Co. Ltd., China) using a microwave digestion system (MDS-6 G, Sineo Microwave Chemistry Technology Co. Ltd, Shanghai, China). The digestion procedure was set as 15 min to 120 °C, 15 min to 180 °C, and 30 min at 180 °C. After cooling, the samples were diluted to 25 mL with deionized water (18.2 MΩ cm, Direct-Q 3, Millipore SAS, France). The total As contents were determined by inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7700x, Japan) with germanium (1.0 μg/mL, Agilent, USA) as an internal standard solution. The polyatomic interference of $^{40}Ar^{35}Cl^{+}$ on *m*/*z* 75 was minimized by a collision/reaction cell. The As concentrations were expressed as μg/g dry weight (dw). Quality assurance was performed by analyzing standard reference material (SRM) BCR627 tuna (Institute for Reference Materials and Measurements, Belgium). Recovery rate from BCR627 was 98.2 ± 3.6 %. Parallel to the samples, deionized water (5 replicates) were processed equivalently to the samples and then analyzed to obtain method blank values for quality insurance/quality control. The AsIII- and AsV- supplemented diet were also digested and the total As

concentrations were shown in Table S1.

2.3. *Arsenic species analysis*

Arsenic species in fish livers were detected using anion exchange high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS; HPLC, Agilent 1260, Tokyo, Japan) following the protocol described by Jia et al. (2018). Briefly, 10 mL of 1% HNO_3 solution was added to the sample, and the mixture was heated for 90 min at 100 °C on a microwave extraction system. The extracted solutions were diluted to 25 mL with deionized water and passed through 0.22 μm millipore filters for As species analysis. SRM BCR627 tuna was utilized for quality assurance. The recovery rates were 88.7-103% for DMA and 89.1-105% for AsB in SRM. Since there was no SRM certified for other four As species (AsIII, AsV, MMA, and AsC), an in-house reference sample was prepared by adding 5 μg/L As standard mixture to fish liver sample containing trace amount of As. The recovery rates of AsC, MMA, AsIII, and AsV were 90.4-102%, 85.9-94.1%, 89.3-99.7% and 90.1-104%, respectively. According to the method by U.S. Environmental Protection Agency (EPA) (2011), the method detection limits (MDL) were calculated on the basis of 3.143 times of the standard deviations of the method blank signals (n = 7). The MDL were 9 μg/kg for AsV, 3 μg/kg for AsIII, 1 μg/kg for MMA, 3 μg/kg for DMA, 2 μg/kg for AsC, 3 μg/kg for AsB, respectively. The method blank was performed throughout the entire sample preparation for each batch. The operating parameters for HPLC and ICP-MS followed the description by Jia et al. (2018).The concentrations of As species in control (basal) and As supplemented diets were also analyzed and the results were shown in Table S1.

2.4. *Biochemical analysis*

The fish livers were washed with cold phosphate-buffered saline (PBS, 0.01 mol/L, PH 7.4), followed by homogenization solely using a tissue homogenizer (TL2010s, DHSBIO) in cold PBS. The homogenates were centrifuged at 9000 × g at 4 °C for 10 min. The obtained supernatants were collected for analysis of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol.

2.5. *Statistical analysis*

Statistical analyses were performed using SPSS version 19.0. Arithmetic mean and standard deviation (SD) in the figures were calculated based on the results from analyses of 7 different fish livers in each test group. The differences of the corresponding values among different treated groups were tested by one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test. Pearson's *r* was utilized to assess the correlations between variables. A probability level (*p* - value) of less than 0.05 was regarded as statistically significant.

3. Results

3.1. *Bioaccumulation of arsenic*

Figure 1 shows the As concentrations in liver over time upon exposure to AsIII and AsV for 40 days. The As concentration in the liver in control group was 0.022 ± 0.004 μg/g, which was much lower than that in exposure groups and not shown in the figure. In both exposure groups, the concentrations of total As increased gradually and reached maximum

values at 30 d, which were 0.853 μg/g and 0.909 μg/g for AsIII and AsV group, respectively. Then the total As fleetly decreased after 30 d and dropped to 0.311 ± 0.065 and 0.465 ± 0.049 μg/g in AsIII and AsV group, respectively. The significantly different As accumulation in liver between AsIII and AsV group was found at 5 and 40 d, with higher As contents found in AsV than in AsIII group. This suggests that the species of dietary iAs affect the As bioaccumulation in fish liver.

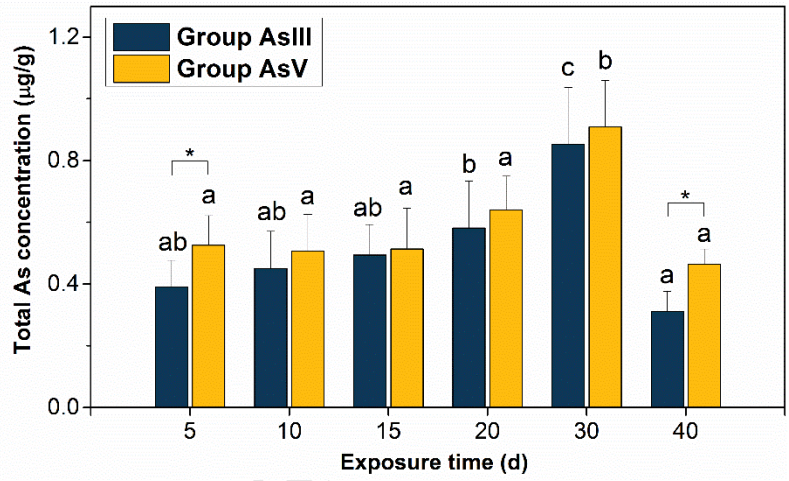

Figure 1. The concentrations of total As (μg/g, dry weight) in livers of *C. auratus* chronically exposed to 50 μg/g dietary AsV and AsIII for 40 d. The data are shown as the means ± SD (n = 7). Different letters represent significant differences at different times in the same treatment group ($p < 0.05$); Asterisk (*) indicates significant difference between AsV and AsIII group at the same exposure time ($p < 0.05$). AsV, arsenate; AsIII, arsenite.

3.2. *Biotransformation of arsenic*

Figure 2 shows the concentrations of the As species in the livers of fish and Figure 3 shows the fractions. AsB was the only species detected in the liver of control fish, with the concentration of 0.020 ± 0.005 μg/g, which was much lower than that in exposure groups and not shown in the figures. In the AsIII group, the concentration and fraction of AsIII species reached the maximum at 5 d (0.084 ± 0.023 μg/g, 31.7%) and reduced gradually over time

and went down to 0.008 ± 0.003 μg/g (2.8%) at 40 d, while a temporary increase was found at 30 d. On the contrary, the contents of MMA, DMA, AsC and AsB increased readily over time and reached the peak value at 30 d and decreased at 40 d. The content of AsV increased to 0.016 ± 0.003 μg/g at 20 d and decrease to 0.008 ± 0.002 μg/g at 30 d and was not detectable at 40 d. The species of AsC and AsV were minor components and the fractions were indicated below 5%. However, the AsB fraction was found increasing steadily during the whole period of exposure. In the AsV group, the As species levels were varied following the similar trend as that in the AsIII group. Nevertheless, the levels of AsIII, AsB, and AsV in AsV group were definitely higher than that in AsIII group.

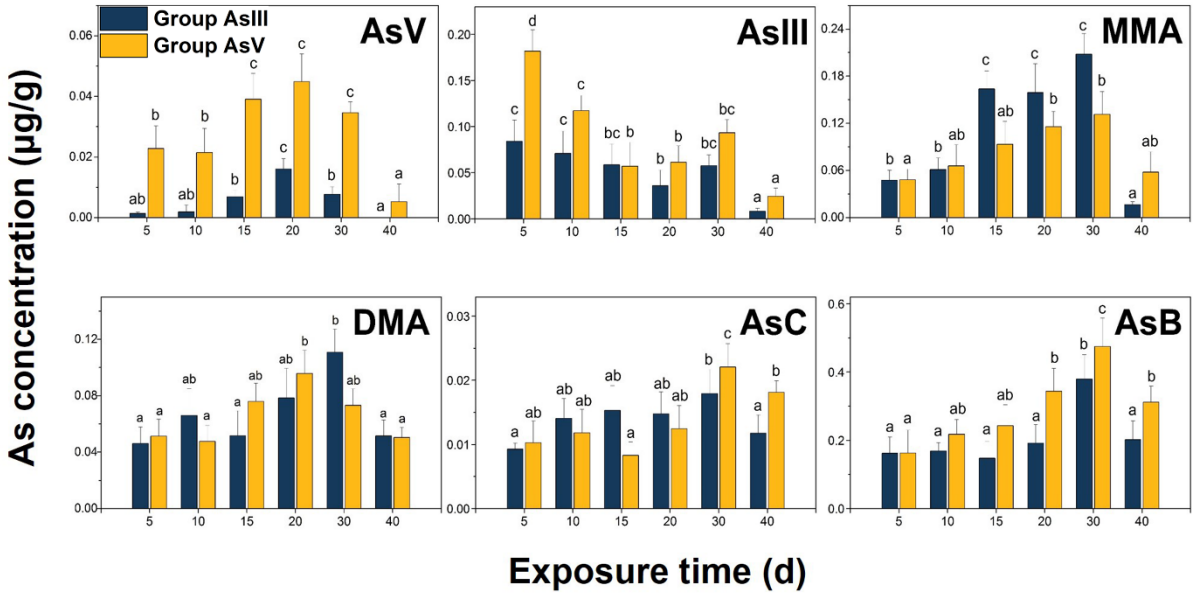

Figure 2. The concentrations (μg/g, dry weight) of different As species in livers of *C. auratus* chronically exposed to 50 μg/g dietborne AsIII and AsV for 40 d. The data are expressed as the means ± SD (n = 7). Different letters represent significant differences at different times in the same treatment group ($p < 0.05$). AsV, arsenate; AsIII, arsenite; MMA, monomethylarsonate; DMA, dimethylarsinate; AsC, arsenocholine; AsB, arsenobetaine.

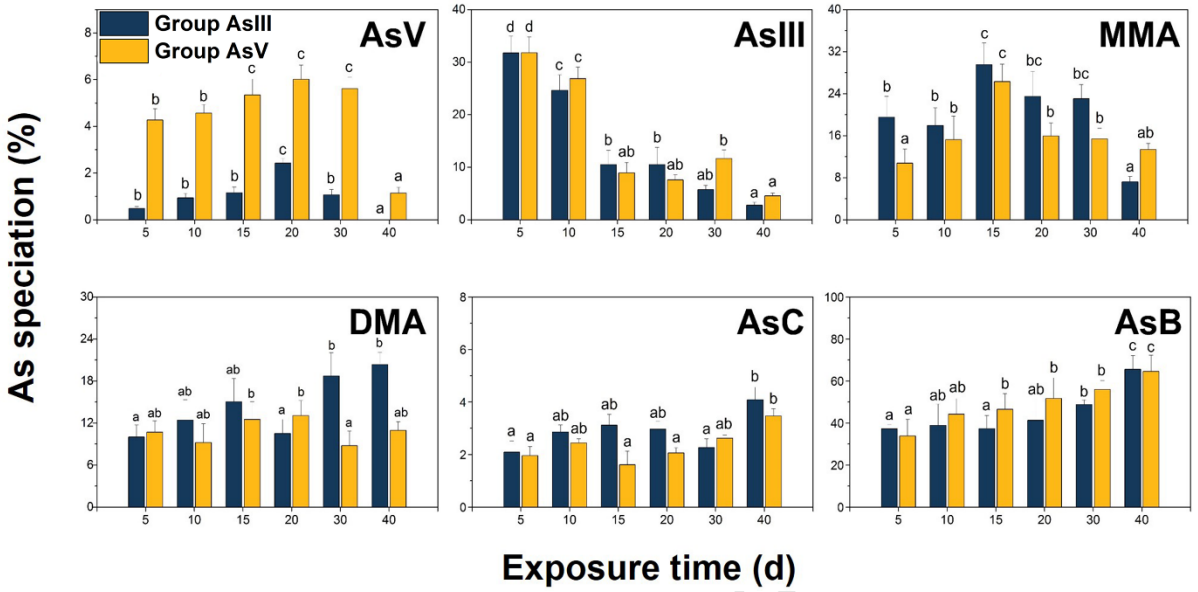

Figure 3. The proportions (%) of different As species in livers of *C. auratus* chronically exposed to 50 μg/g dietborne AsIII and AsV for 40 d. The data are expressed as means ± SD (n = 7). Different letters represent significant differences at different times in the same treatment group ($p < 0.05$).

To precisely reflect the methylation degree of iAs, the primary methylation index (PMI) and the secondary methylation index (SMI) were calculated to assess the As biomethylation ability of AsV- and AsIII- exposed fish (Liu et al., 2017). PMI and SMI are the ratio of MMA + DMA to total As and DMA to MMA + DMA, respectively. As shown in Figure 4, the two iAs exposure groups showed similar variation trend on PMI, with slightly higher in AsIII group than that in AsV group. For the SMI, there was no significant difference during the first 30 days between the two groups. At 40 d, however, the SMI was significantly higher in AsIII group than that in AsV group. Therefore, the original iAs species had a minor effect on the primary methylation process but greatly influenced the secondary methylation process in the late stage of exposure.

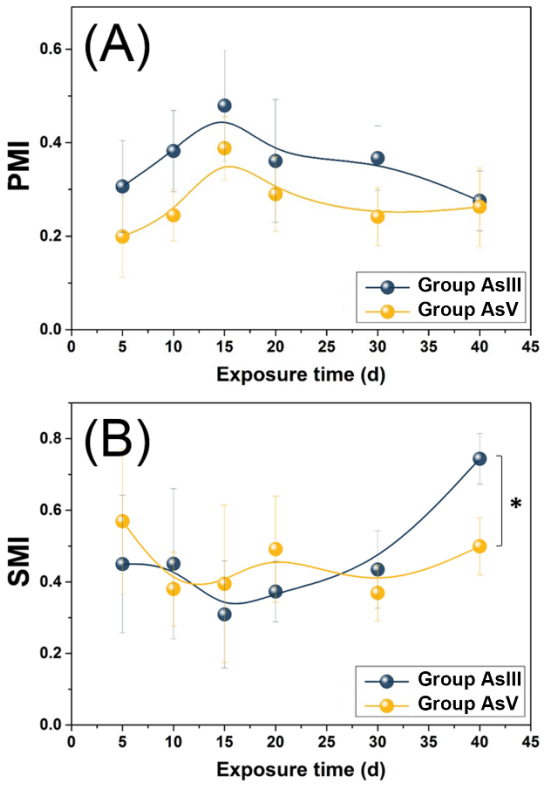

Figure 4. The PMI (A) and SMI (B) in livers of *C. auratus* chronically exposed to 50 μg/g dietborne AsIII and AsV for 40 d. All values are means ± SD (n = 7). Asterisk (*) represents significant difference between the As exposure groups at the same exposure time ($p < 0.05$).

The correlations between the concentrations of respective As species and the total As in liver was further analyzed (Figure 5). The concentration of iAs species rose until reaching a plateau as the total As increasing, while, organic As (OrgAs, MMA + DMA + AsC + AsB) was positively correlated with the total As. The correlation coefficient between AsB/OrgAs levels and total As levels was 0.41/0.67, indicating that the OrgAs species, especially AsB, contributed more to the total As accumulation in fish liver.

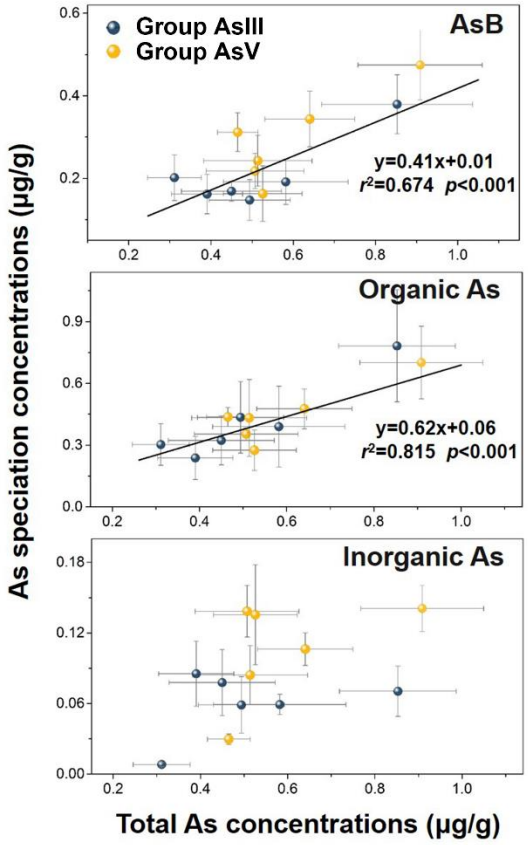

Figure 5. The correlation between the total As concentrations and AsB, the organic As, and the iAs concentration in livers of *C. auratus* chronically exposed to 50 μg/g dietborne AsIII and AsV for 40 d.

3.3. *Oxidative stress induced by inorganic arsenic exposure*

The four oxidative biomarkers in liver of crucian carp were all changed during the exposures (Figure 6). The SOD activities at 15 d and 20 d were significantly upregulated by AsIII and AsV exposure. The CAT activities were significantly enhanced at 15 d, while a decrease was observed in AsV group at 10 d. The GSH level in AsIII group at 5 d was significantly higher than that in the control group, however, there was no significant change in AsV group compared with the control group. The MDA levels significantly rose at 15 d and then recovered from 20 d to 40 d in the AsIII group and there was no significant variation in the AsV group compared with the control group.

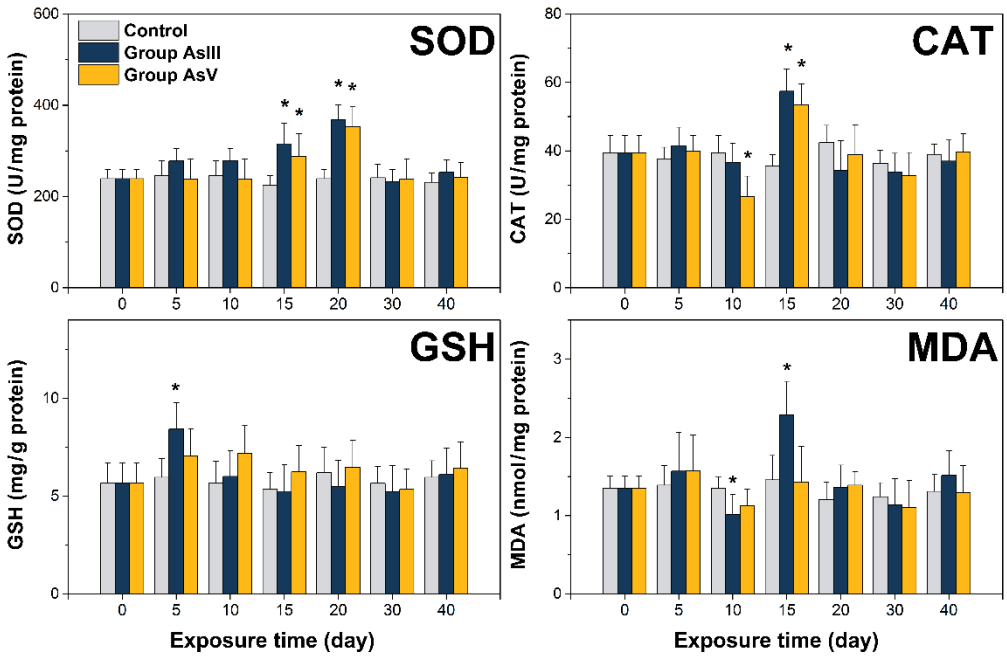

Figure 6. SOD, CAT activities and GSH, MDA contents in livers of *C. auratus* chronically exposed to 50 μg/g dietborne AsIII and AsV for 40 d. Values are means ± SD (n = 7). Significant differences versus control are marked with * ($p < 0.05$).

As shown in figure 7, the SMI level was positively and negatively correlated with the MDA level, respectively, in AsV and AsIII groups at 5 d, indicating that the dimethylation might play important roles in the effect of dietary iAs. No correlation was found in treatment groups of other time points. In addition, there were no apparent correlations between levels of MDA and MMA (or MMA%), DMA (or DMA%), AsV, AsIII, iAs, total As, or PMI in each of exposure treatment (figures not shown). Possibly, different As species have different pharmacokinetic properties. SMI was thus proved to be a consistent indicator of As biotransformation ability than other ratio or species levels (concentrations or fractions). This may be due to that MMA and DMA have similar biological half-lives in liver of crucian carp.

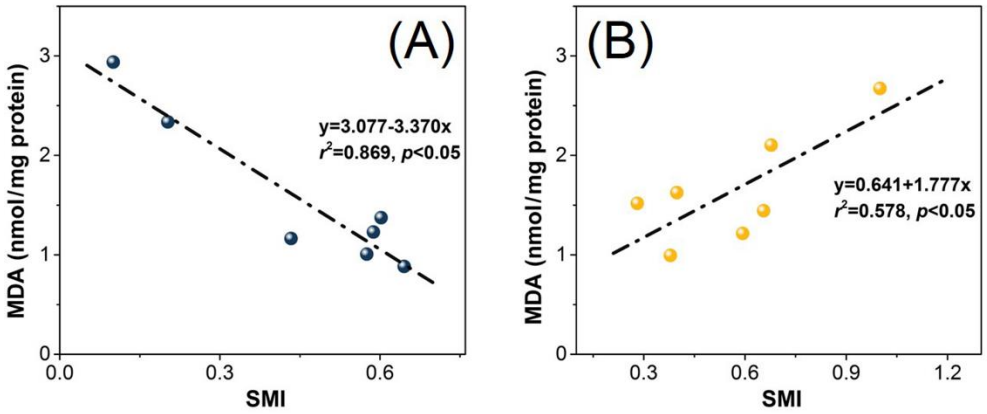

Figure 7. Correlation between SMI and MDA in livers of *C. auratus* after exposure to AsIII (A) and AsV (B) for 5 days.

4. Discussion

4.1. *Adaptive responses of fish to iAs exposure*

Liver is a major site for elimination and biotransformation of As (Twaddle et al., 2018). In the present study, total As accumulation in liver increased readily over time and then decreased quickly under dietary iAs exposure. These were companioned by the continuous increase of the fraction of benign AsB and the decrease of the fraction of toxic iAs (AsIII + AsV). This demonstrated that the biotransformation process of iAs in the liver of fish may sever as a detoxification process that can adapt to the external environment. Fish are relatively resistant to the dietary iAs at this environmental relevant concentration but still undergo temporary biotransport and bioconversion processes at the initial exposure stage. Consistently, Pedlar et al. (2002) found a notably reduction of As content in liver of lake whitefish after 30 d of As dietborne exposure. The time-dependent alteration of As contents and As species fraction in fish were rarely reported in dietborne experiment but were very common in waterborne. Chen et al. (2018) found the decreasing in fractions of the AsV and

AsIII in medaka (*Oryzias mekongensis*), respectively, after 100 μg/L AsIII and AsV exposures for 28 d, accompanied by an increasing in the fraction of AsB. However, Bears et al. (2006) found an elevated level of total As and a lower relative proportion of AsB versus toxic As species in liver of killifish (*Fundulus heteroclitus*) after exposure to iAs. AsB has been considered as the terminal species for iAs biotransformation and was the predominant species of As in marine organisms (Zhang et al., 2018). In our study, the AsB percentages was found higher in AsV-fed fish than that in AsIII-fed fish from 10 d to 30 d, while the difference disappeared at the end of exposure. Similar results were also reported by Zhang et al. (2016a), where they found a higher fraction of AsB in liver of marine rabbitfish after 400 μg/g AsV exposure than AsIII for 21 d, however, no difference was found after 42 d. Therefore, it is necessary to investigate the temporal profiles of iAs metabolism under chronic exposure when study the influence of dietborne iAs, since the responses to iAs might be missed if the evaluation was just based on the short-term of exposure.

The adaptive responses were also reflected by the enhanced antioxidative defense in the liver of fish before 20 d and the disappearance of oxidative stress at the late stage. The enhanced GSH concentration and CAT and SOD activities suggest that both nonenzymatic and enzymatic antioxidants were activated to defense the iAs induced oxidative stress. Despite of this, the over accumulation of MDA in the liver of fish at 15 d of exposure to AsIII was still observed, suggesting oxidative damage occurred. The SOD activity still increased at 20 d to continuously defend the oxidative stress. Neither the levels of antioxidants nor the MDA were affected after 20 d, suggesting the oxidative damages was eliminated. Superoxide radicals produced by As uptake were transformed to H_2O_2, which was catalyzed by SOD

(Amuno et al., 2020). The produced H_2O_2 could be consequently decomposed to H_2O by the action of CAT. Both anti-oxidizing processes directly protect body against the harmful ROS produced by iAs in fish (Bagnyukova et al., 2007).

GSH plays a vital role in the detoxification of uptake As in liver of fish. In addition to defense the oxidative stress, GSH acts as the electron donor for AsV conversion to AsIII species, and combines with trivalent arsenicals *eg.* AsIII, MMAIII, and DMAIII to facilitate As clearance and reduce As toxicity (Scott et al., 1993). Moreover, As metabolism in the liver could directly affect the As species accumulation in the muscle. According to our study outcome from fish muscle, the AsIII concentration and fraction in AsV group sharply increased and reached a maximum at 10 d (1.257 ± 0.406 μg/g and 79.3 ± 6.4%) and then decreased gradually from 10 to 40 d (unpublished results). There might be two reasons for the transient rise of AsIII level in muscle: (a) Trivalent arsenicals (AsIII, MMAIII and DMAIII) had a high capability to combine with GSH, thus exhausting the GSH levels in liver cell at the initial stage of exposure (Scott et al., 1993). The consumption and inadequate supply of GSH in liver could not make sure a timely clearance of the massive AsIII reduced from AsV. This partly resulted in large amount of AsIII being transported to muscle and sequestered there. (b) The process of GSH conjugating with As was catalyzed by glutathione-S-transferase (GST) to produce As-GSH compound which could be eliminated out of the cell (Moreira et al., 2016). However, the increased activity of GST could act as an inhibitor of multidrug resistance-associated proteins (MRP), which are proposed to transport a diverse range of anionic substrates, including As-GSH conjugates, out of cells. Thus the overexpression of GST might weaken the As elimination. Shaw et al. (2007) reported that the

increased expression of MRP in liver was responsible for transporting As-GSH conjugate out of cells to reduce body As levels of As-resisted fish *Fundulus heteroclitus*. The complication brought by such processes of As metabolic is a balance between detoxification, both biochemically and by elevating elimination from fish body, and activation to reactive GSH capable of interactions with metabolically available As. The relationship between the transport protein and toxicokinetic process needs to be studied further.

4.2 *Arsenic species dependent correlation of arsenic dimethylation with oxidative damage*

The toxicity of As is proposed to be linked to the imbalance between antioxidant and prooxidant that leads to oxidative stress (Tuulaikhuu et al., 2016). However, whether the methylation is to potentiate or detoxify As toxicity remains an on-going discussion. MDA is a biomarker of oxidative damage due to the sensitivity of membranes to be attacked by ROS (Capolupo et al., 2016). The association analyses of MDA level and iAs metabolites in liver can help to better verify the toxic potential of iAs in fish. In the present study, the increase of As dimethylation level was associated with increasing MDA level in liver of AsV group, suggesting that As dimethylation induced oxidative stress in the early stage of exposure. There is considerable evidence that iAs exposure induce the generation of ROS and lipid peroxidation in fish (Bhattacharya and Bhattacharya, 2007). However, continuous As exposure was commensurate with elevated enzymatic antioxidant (SOD and CAT) and nonenzymatic antioxidant (GSH) defense systems whose activation reduced the generation of ROS and MDA (Sarkar et al., 2014). The present result of the decreased MDA level in our study substantiated that crucian carp could activate corresponding defensive systems to counteract the oxidative stress caused by AsV exposure. In AsIII group, however, the increase

of As dimethylation level was associated with the decreasing MDA level in liver, indicating that As dimethylation could relieve the oxidative damage. The opposite correlationship was also reported in epidemiological studies. Some data showed that poor efficiency of As dimethylation (indicated by low DMA/MMA ratio or low percent of DMA) in urine is connected with the increased risk of As-related diseases; from the other data, however, the positive association was observed between higher DMA% or SMI in urine and increased risk of As-diseases (Khan et al., 2020). Dopp et al. (2010) reported that the methylating cells (hepatocytes) were more prone to As-induced cytotoxicity than the non-methylating cells (urothelial cells). Chen et al. elucidate that the MDA levels in marine medaka changed in a similar trend as the body iAs contents under iAs waterborne exposure, thus speculated that iAs accumulation caused oxidative stress in the initial stage of exposure (Chen et al., 2019). From our result, the increased iAs bioaccumulation was not found to induce the increase of MDA levels in both iAs groups. In most organisms, the liver carries out the most function of As biotransformation. For pentavalent As, the methylation is coupled with the reduction of pentavalent As to trivalent As, as indicated in the following reactions: AsV + 2e → AsIII + Me+ → MMAV +2e → MMAIII + Me+ → DMAV +2e → DMAIII (Zhou and Xi, 2018). The pentavalent methylated As (MetAs), MMAV and DMAV, were not cytotoxic in a tested concentration range of 0.5-5 mmol/L in different cell lines (Dopp et al., 2010). In all test systems, DMAIII was the most cytotoxic species (LC 50: 9-12 μmol/L) followed by MMAIII (LC 50: 12-18 μmol/L), AsIII (LC 50: 130-170 μmol/L) and AsV (LC 50: 500-1500 μmol/L). Therefore, the secondary methylation could actually decrease the toxicity by eliminating the toxic MMAIII, however, also generate toxic DMAIII. Rehman et al. (2014) reported that the

two methylation metabolites MMAIII and DMAIII predominantly caused DNA damage via oxidative stress in HL-60 cells, while iAs species did not show any related effect. Recently, Twaddle et al. (2018) reported that high levels of MMAIII were evident accumulated in liver of CD-1 mice dosing with AsIII, while the accumulation was just occurred at the early time point of exposure. The biochemistry and modes of toxic actions of dietary AsIII and AsV to organisms are different. From the present results, the process of DMAV bio-reducing to DMAIII might be predominant in AsV-fed fish at the initial stage of exposure, compared with the process of MMAIII bio-oxidation to DMAV. In AsIII group, however, the later process could be predominant. Therefore, the rate of MMA methylation to DMA is very important in determining whether the methylation process is a poisoning mechanisms or simply a biotransformation process of fish. The SMI index calculated as DMA/(MMA + DMA) could act as a consistent indicator to depict the relative production of toxic trivalent MetAs species compared with non-toxic pentavalent MetAs species in fish tissues. The effectiveness of the detoxification would depend on both the biomethylation ability of fish and their anti-oxidation ability based on a wide range of nonenzymatic antioxidant and enzymatic antioxidants. These different effect between AsIII and AsV group reflected complex relationships involving biotransformation pathway, toxicodynamics, and toxicokinetics, and raise various issues which need consideration in future work if these differences are to be understood.

5. Conclusions

This study investigated the dynamic variations of bioaccumulation, biotransformation, antioxidative response and oxidative damage in freshwater fish *C. auratus* chronically

exposed to dietary AsV and AsIII (50 μg/g). This study found that acclimated physiology occurred to increase the resistance to As toxicity during chronic iAs exposure, which was associated with the reduction of total As and iAs bioaccumulation and an increase of As biotransformation to a less-toxic form (AsB). The AsV and AsIII dietary exposure could both activate antioxidant enzymes activities and only the dietary AsIII induced significant oxidative damage at 15 d. However, the oxidative stress mitigated as the exposure prolonged, which might be related to the absorption and elimination processes as well as the internal As biotransformation in fish to acclimate the environment. At the initial exposure stage, the dimethylation activity could be a detoxification mechanism or a poisoning process, which was found to be dependent upon the exposure iAs species. Therefore, our study suggests that the responses in fish to chronic iAs dietborne exposure may be missed if the assessments only based on total As or iAs burdens, more effective and sensitive indicators should be developed when evaluating the chronic toxicity of As exposure.

Supporting information

Total As, As species concentrations (μg/g, dry weight) and fractions (%) in basal and As-supplemented artificial diets.

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Declaration of competing interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author contributions

Di Cui: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Project administration, Data Curation, Writing - Original Draft, Writing - Review & Editing.

Peng Zhang: Writing - Review & Editing, Validation, Funding acquisition, Visualization.

Haipu Li: Conceptualization, Resources, Supervision, Funding acquisition, Project administration, Writing - Review & Editing.

Zhaoxue Zhang: Methodology, Writing - Review & Editing, Data Curation.

Yang Song: Investigation, Methodology, Validation.

Zhaoguang Yang: Resources, Supervision, Funding acquisition.

Highlights

- Arsenobetaine became the predominant species in fish after dietborne arsenic exposure.
- As acclimation involved both reduced As accumulation and increased As transformation.
- The toxic action of As dimethylation was dependent upon the pristine As species.