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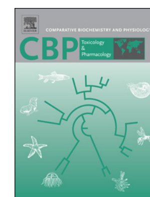

Acute ammonia toxicity in crucian carp *Carassius auratus* and effects of taurine on hyperammonemia

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ABSTRACT

The four experimental groups were carried out to test the response of crucian carp *Carassius auratus* to ammonia toxicity and taurine: group 1 was injected with NaCl, group 2 was injected with ammonium acetate, group 3 was injected with ammonium acetate and taurine, and group 4 was injected with taurine. Fish in group 2 had the highest ammonia and glutamine contents, and the lowest glutamate content in liver and brain. Serum superoxide dismutase (SOD), glutathione (GSH) activities, red cell count (RBC), white cell count (WBC), lysozyme (LYZ) activity, complement C3 content of fish in group 2 reflected the lowest, but malondialdehyde content was the highest. Importantly, serum SOD and GSH activites, RBC, WBC, and LYZ activity, C3, C4 and total immunoglobulin contents of fish in group 3 were significantly higher than those of fish in group 2. This study indicates that ammonia exerts its toxic effects by interfering with amino acid transport, inducing ROS generation, leading to malondialdehyde accumulation and immunosuppression of crucian carp. The exogenous taurine could mitigate the adverse effect of high ammonia level on fish physiological disorder.

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1. Introduction

Hyperammonemia is a key factor involved in the pathogenesis of hepatic encephalopathy (HE) and brain edema, two serious neurological complications of acute liver failure in mammals (Mireille et al., 2007). A previous study surmised that the symptoms of fish ammonia poisoning might be similar to the action of ammonia in mammals during hyperammonemia (Smart, 1978): Under normal physiological conditions, endogenous or exogenous ammonia are detoxified in the liver. However, when the liver fails, ammonia rapidly accumulates in the circulation, freely crosses the blood-brain barrier and accumulates in the brain, result in astrocyte swelling, even death (Felipo and Butterworth, 2002). However, recent studies have confirmed that some marine fish can tolerate high levels of brain ammonia (Chew et al., 2005; Ip et al., 2005). It is possible that the defense mechanism against ammonia toxicity in fishes is different from that in mammals. Ip et al. (2005) reported that glutamine accumulated to exceptionally high levels in brains of mudskippers *Periophthalmodon schlosseri* (29.8 μmol g^{-1}) and *Boleophthalmus boddaerti* (12.1 μmol g^{-1}) without causing death, it can be concluded that these two mudskippers could ameliorate those problems associated with glutamine synthesis and accumulation from hyperammonemia. Sun et al. (2012) found that ammonia significantly restrained the antioxidant system in bighead carp *Hypophthalmythys nobilis*. Chen et al. (2011) reported that the immune response of tilapia *Streptococcus iniae* was restrained by ammonia exposure. To the author's best knowledge, ammonia poisoning in fish has been shown follow multifactorial pathogenesis, the link among free amino acid metabolic disorder, oxidative stress and immunosuppression remains undefined (Ip et al., 2005; Hegazi et al., 2010).

Taurine (2-aminoethanesulfonic acid) is an organic acid which was first described from ox bile (Guillaume and Allen, 2015). In mammals, taurine is a potent regulator of proinflammatory and immune response, it has been linked to a number of health benefits, including tissue repair, antioxidation, immunoregulation, alleviation of metal toxicity, osmoregulation and neuromodulation (Gulyasar et al., 2010; Motawi et al., 2007; Nakamura et al., 1993; Zhang et al., 2010). Chepkova et al. (2006) reported that taurine may rescue hyperammonemia of mouse by improving mitochondrial function, regulating glutamine synthetase activity, reducing ammonia and glutamine accumulation. Hyperammonemia is arguably the most specific symptom of taurine deficiency in mammal, but it has not been reported in fish species. In recent years, fish hyperammonemia has become a more and more serious problem, due to intensive aquaculture systems can easily cause ammonia stress (Randall and Tsui, 2002; Sun et al., 2012). A recent study in our lab found yellow catfish *Pelteobagrus fulvidraco* fed all-plant protein diet supplemented with taurine could mitigate the adverse effect of hyperammonemia on fish survival (Li et al., 2016).

Crucian carp *Carassius auratus*, an omnivorous fish, is a freshwater fish that inhabits the lakes, rivers and reservoirs in various countries in Asia and Europe, and is the most important cultivated species in China. To

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our knowledge, no information is available concerning the effects of taurine on physiological of crucian carp of hyperammonemia up to now. The experiments with such a design would enable us to test the following hypotheses: (1) acute ammonia toxicity may negatively affects free amino acid metabolism, antioxidant enzymes activities, hematology and immune response in crucian carp, and (2) the hyperammonemia of crucian carp can be mitigated by exogenous taurine.

2. Materials and methods

2.1. Animals and experimental conditions

Crucian carp were purchased from a fish farm (Huzhou, China), and were acclimated to laboratory conditions for 14 d feeding commercial feed before experiments. Fish (40.3 ± 4.33) g was randomly distributed into 12 rectangular plastic tanks (300-L) with 30 fish per tank in triplicate. No attempt was made to separate the sexes. All tanks were supplied with dechlorinated tap water with a daily exchange rate of 1/3 tank volume. Water was continuously aerated using air stones. During the trial, the water temperature ranged from 27 °C to 29 °C, dissolved oxygen 7.81 ± 0.13 mg L^{-1}, pH .4–6.6, nitrite <0.5 mg L^{-1}, and 12 h light and 12 h dark.

2.2. Experimental designs and sampling

To determine the 96 h LC_{50} of ammonium acetate injected into the peritoneal cavity, five groups of crucian carp ($N = 10$) were anesthetized for approximately 10 min in tricaine methanesulfonate (MS-222) at 120 mg L^{-1} and injected intraperitoneally with 6, 9, 12, 15 and 18 μmol ammonium acetate g^{-1} fish. Control group ($N = 10$) was injected with 0.9% NaCl. Mortality was monitored during the subsequent 96 h. Fish was regarded as dead when there was no respiratory activity and no reaction to mechanical stimulation. The LC_{50} level (9 μmol ammonium acetate g^{-1} fish) was then used for the experimental challenge.

The four experimental groups were coded as group 1 (control), 2, 3 and 4, respectively: control group (group 1) was injected intraperitoneally with 0.9% NaCl; group 2 was injected intraperitoneally with a LC_{50} dose of ammonium acetate (9 μmol g^{-1} fish); group 3 was injected intraperitoneally with 100 μg taurine g^{-1} fish (Chepkova et al., 2006; Zielinska et al., 2003) 10 min prior to the injection with a LC_{50} dose of ammonium acetate; group 4 was injected intraperitoneally with 100 μg taurine g^{-1} fish. The experiment continued for 96 h (Ip et al., 2005).

At the end of trial, all fish were anesthetized with MS-222 for counting. Blood (3 fish $tank^{-1}$) was collected from caudal vasculature using heparinized syringes for hematological assays. Another blood (3 fish $tank^{-1}$) was collected using nonheparinized syringes, and serum was obtained by centrifugation (×4500 *g*, 4 °C) and stored at −80 °C for serum constituent, antioxidant enzyme activity and immunological assays. After being bled, liver and brain of 6 fish were removed for ammonia and free amino acid content analysis.

2.3. Ammonia and free amino acid content analysis

Based on the report of Ip et al. (2005), the frozen brain and liver were weighed, ground to a powder in liquid nitrogen and homogenized three times in 6% trichloroacetic acid at 24,000 *g* for 20 s each. The homogenate was centrifuged at 10,000 *g* at 4 °C for 15 min. Ammonia analysis was determined following the methods of Bergmeyer and Beutler (1985). Freshly prepared NH_4Cl solution was used as the standard for comparison.

The frozen brain and liver were added to 5% perchloric acid solution and the sample was well mixed using a homogenizer. The suspension was centrifuged at 2500 rpm for 10 min. The extract was used for the free amino acid analysis. Free amino acid were analyzed with a Shumadzu LC-20AD amino acid analysis system (Kyoto, Japan), equipped with a Waters ACQUITY UPLC BEH C18 column (San Antonio, USA), according to the methods of O'Loughlin et al. (2015). Results were expressed as μg g^{-1} wet mass tissue.

2.4. Antioxidant enzyme activity and lipid peroxidation assays

The levels of enzyme activity and lipid peroxidation were measured with commercial assay kits (Nanjing Jiancheng Institute, Nanjing, China) in accordance with the instructions of the manufacturer. The assays are briefly described as follows: Total superoxide dismutase (SOD) activity was determined following the methods of Beauchamp and Fridovich (1971). One unit of SOD activity was calculated using the amount of superoxide dismutase required to inhibit the reduction of nitrobluete trazolium by 50%. Catalase (CAT) activity was determined by measuring the decrease in H_2O_2 concentration (Aebi, 1984). One unit of CAT activity was defined as the amount of CAT required to transform 1 μmol of H_2O_2 per min. The glutathione activity was determined color metrically at 412 nm with a spectrophotometer following reaction with 5, 5-dithiobes-(2-ni-trobenzoic acid) (DTNB) (Jollow et al., 1974). Lipid-peroxidation levels were determined based on the malondialdehyde level generated by oxidizing fatty acids. In the presence of thiobarbituric acid, malondialdehyde started producing colored thiobarbituric-acid-reacting substances (TBARS) that were measured at 532 nm (Buege and Aust, 1978).

2.5. Hematology assays

Red blood cell, white blood cell and hemoglobin were analyzed following the procedure described by Lim et al. (2009). Serum total protein, albumin, globulin, total cholesterol, triglyceride and glucose according to the method described by Wu and Shang (2006) using Hitachi 7600–110 automatic chemistry analyzer (Hitachi Ltd., Tokyo, Japan).

2.6. Immune parameter assays

Lysozyme activity was determined through the turbidimetric method (Hultmark et al., 1980) using a lysozyme detection kit (Nanjing Jiancheng Institute, Nanjing, China). The assay was based on lysis of lysozyme-sensitive Gram-positive bacterium *via* the lysozyme present in serum. Natural complements C3, C4 and total immunoglobulin were assayed using a commercial kit (Zhejiang Elikan Biological Technology Co., Ltd., Wenzhou, China). The methods are described by Wu and Shang (2006). The above commercial reagent supplied in the test kit was added to each well of the microtitre plate at 300 μL $well^{-1}$. Then, 3 μL of serum was added to each well. Samples were incubated at 37 °C for 10 min and subsequently estimated by measuring the optical density of the supernatant at 340 nm on a spectrophotometer.

2.7. Statistical analyses

Assayed parameter values were presented as means ± standard errors of the mean (S.E.M.). All variables were assessed using one-way ANOVA. If there was a significant *F*-test, subsequent comparisons of treatment means were performed using the Duncan's Multiple Range test. The significance level was set at $P < 0.05$. All analyses were performed using SPSS 18.0.0 (Chicago, USA) for Windows.

3. Results

3.1. Survival rate

Fish survival rate was affected by ammonium acetate. The lowest survival rate was found in group 2 ($P < 0.05$; Table 1), no significant differences were found among other groups ($P > 0.05$).

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