Modification of saltwater stress response in *Cyprinus carpio* (Linnaeus, 1758) pre-exposed to pesticide indoxacarb

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**A R T I C L E   I N F O**

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**A B S T R A C T**

To evaluate the effects of indoxacarb on saltwater stress response in *Cyprinus carpio*, the fish were pre-exposed to indoxacarb (0, 0.75, 1.5 and 3 mg/L denoted as CP, 0.75IT, 1.5IT and 3IT, respectively) for 21 days and then released to saltwater. A negative control (CN) group was included (the fish were held in indoxacarb-free water for the entire experiment). The fish were sampled immediately (0 h) and 24, 48, and 72 h after the salinity exposure for the analysis of plasma cortisol, glucose and sodium, chloride, potassium and calcium levels. All fish pre-exposed to 3 mg/L indoxacarb, died after the first day of salinity challenge. CP showed typical cortisol response after the salinity challenge, but, cortisol response of the fish pre-exposed to indoxacarb (0.75IT and 1.5IT) was blocked. Plasma glucose increased significantly in all groups compared to the CN; however, this elevation had no consistent trend in 0.75IT and 1.5IT which indicated interference in glucose response due to indoxacarb exposure. Plasma sodium increased (compared to CN) in all groups after the salinity challenge. However, elevation in plasma chloride and potassium was significantly different among the groups and the indoxacarb-treated fish showed slightly sooner ionic disturbance. The results clearly indicate that indoxacarb impairs stress response of *C. carpio* and the fish may not be able to respond normally to additional stressors, which threatens their survival.

1. Introduction

Water pollution is a widespread problem in many aquatic environments (Hori et al., 2008). Xenobiotics enter water bodies due to agricultural, industrial and municipal activities. Upon entrance, xenobiotics cause several physiological and pathological alterations in fish which may weaken the animal and decrease its survivorship (Hedayati et al., 2014, 2016; Hoseini et al., 2014, 2016b; Hoseini and Tarkhani, 2013).

Aquatic animals are exposed to environmental stressors and react to these stressors with a variety of physiological responses (Barton, 2002). These physiological responses restore the fish body homeostasis and guarantee the fish survivorship. Osmotic stress is one of the common stressors in aquatic environment which threatens fish life (Assem and Hanke, 1981). It causes hydromineral imbalance and increases energy demand by elevation in cortisol secretion and glucose mobilization (Hoseini and Hosseini, 2010). Cortisol is the main stress hormone in fish which has important role in hyperosmotic stress tolerance. Cortisol provides demanded energy for osmoregulation, increases chloride cell number and size, and stimulates Na⁺,K⁺-ATPase activity; all these changes help the fish to restore hydromineral balance (Hoseini and Hosseini, 2010; Madsen, 1990; McCormick, 1990). Xenobiotics may deteriorate fish stress responses (Barton, 2002; Cericato et al., 2009). Pacheco and Santos (1996) and Nascimento et al. (2012) suggested that exposure to xenobiotics cause fish lose their capacity to elevate cortisol in response to additional stressors. Several field studies have confirmed the adverse effect of prolonged exposure to agrichemicals on wildlife hypothalamus–pituitary–interrenal axis (HPI) (Hontela, 1998).

Indoxacarb is an applicable pesticide, organophosphate alternative, to manage some crops such as tobacco and cotton in the north of Iran (Malekzadeh and Javadzadeh, 2002; Mohaghhegh Naishabouri et al., 2009) and also is used to control lepidopteran larvae in tomato, lettuce, bean and some other crops (Ghelichpour et al., 2017; Mirghaed and Ghelichpour, 2015). Although, indoxacarb is moderately hydrophobic with a low water solubility and short aqueous photolysis half-life it is classified as “moderately to very highly toxic” to freshwater and estuarine fish (Moncada, 2003). Little is known about this pesticide effects on fish species. Indoxacarb is used in the north of Iran for pest control, near the Caspian Sea, where common carp inhabits; thus it is
necessary to investigate toxic effects of indoxacarb on common carp. Previous studies showed that indoxacarb is toxic in common carp and causes histopathological damages, thyroid hormones’ interference and suppressed protein synthesis (Ghelichpour et al., 2017; Mirghaed and Ghelichpour, 2015). However, there is no study about indoxacarb effects on osmotic stress tolerance in fish. It is important to investigate indoxacarb effects on common carp osmotic stress responses, because this species may be exposed to both osmotic stress and indoxacarb toxicity. In the present study, the effects of long term indoxacarb exposure were investigated on osmotic stress response in common carp.

2. Materials and methods

2.1. Fish acclimation and experimental design

Common carp, weighing 45.2 ± 5.81 g were obtained from the Sijawal Fish Reproduction Center (Golestan province, Northeast Iran). The fish were randomly assigned to fifteen 300 L tanks with 28 fish per tank and acclimated during 7 days. The physicochemical parameters of the water were constantly monitored in the acclimation period and the experiments (temperature = 23 ± 1.27 °C; pH = 8.07 ± 0.25; dissolved oxygen = 7.14 ± 0.84 mg/L; conductivity = 4630 ± 55 μS cm−1; salinity = 2.63 ± 0.15 g L−1; hardness = 300 ± 17.5 mg/L (as CaCO3); alkalinity = 350 ± 20.3 mg/L (as CaCO3) and calcium = 110 ± 11.7 mg/L). During the acclimation, the fish were fed 1.5% body weight once a day with commercial carp feed (Mazandaran Animal & Aquatic Feed Co., Sari, Mazandaran, Iran) under continuous aeration condition and 75% water exchange daily.

After the acclimation week, the tanks were divided into three main groups: 1) indoxacarb treatment group = nine tanks (3 replicate tanks per treatment) were exposed to three different indoxacarb (Hef Iran Co. Tehran, Iran) concentrations [0.75 mg/L (0.75IT), 1.5 mg/L (1.5IT) and 3 mg/L (3IT)] followed by a salinity challenge, 2) positive control (CP) group = three tanks were held in indoxacarb-free water followed by the salinity challenge. A negative control (CN) was assigned too. Experimental design is shown in Fig. 1. The indoxacarb concentrations were chosen according to 96-h-LC50 (0.75 mg/L as 5% of the LC50, 1.5 mg/L as 10% of the LC50 and 3 mg/L as 20% of the LC50). The indoxacarb exposure period was 21 days. During this period, the tanks’ water was daily exchanged (75%) by clean-water. Appropriate indoxacarb amount was added to each tank to maintain the pesticide water was daily exchanged (75%) by clean-water. Appropriate indoxacarb amount was added to each tank to maintain the pesticide

2.2. Analysis

Water temperature, dissolved oxygen, electro-conductivity, salinity, and pH were measured daily during the experiment by Hach HQ40d portable apparatus (Loveland, Colorado, USA). Total hardness, alkalinity, and calcium were determined using photometer (Wagtech 7100, Berkshire, UK). Plasma glucose levels were determined spectrophotometrically by glucose-oxidase method using Pars Azmun kits (Tehran, Iran). The kit detection limit is 5–400 mg/dL. Calcium levels were determined spectrophotometrically by ARSENazo III method using Pars Azmun kits (Tehran, Iran). This kit detection range is 1–20 mg/dL. Plasma chloride levels were determined spectrophotometrically by mercuric cyanate method using Zist Shimi kit (Tehran, Iran). This kit is suitable for detection in 2–200 mEq/L. Appropriate dilution was performed by distilled water when necessary. Plasma cortisol was determined by solid phase ELISA method based on competition principle using a commercial kit (IBL, Gesellschaft für Immunchemie und Immunbiologie, Hamburg, Germany). The test performed on microplate reader and detection limit was 0.05–800 ng/mL. cortisol was determined in duplicate and mean was used for statistical analysis. Plasma samples were assayed for sodium and potassium using a flame photometer (SEAC, Florence, Italy).

2.3. Statistical analysis

Data had normal distribution (Shapiro-Wilk test). Data were analyzed with a one-way ANOVA and Tukey test. P < 0.05 was considered as significance. Data were presented as mean ± SD. All analyses were performed using SPSS software (v.22).
3. Results

All fish in 3IT groups died during the first day of the salinity challenge thus were omitted from the experiment. Plasma cortisol levels were significantly different among the treatments. Indoxacarb treatment had no significant effect on the pre challenge (0 h) plasma cortisol levels. Cortisol values were significantly affected by salinity challenge alone in the CP group, as it increased 24 h after the challenge and continued until 48 h. However, increase in plasma cortisol levels of the 0.75IT group occurred 48 h after the salinity challenge, with a delay in comparison to the CP group. After salinity challenge, no significant difference in plasma cortisol levels was observed in the 1.5IT group compared to the CN (Fig. 2).

Fig. 3 shows no significant effects of indoxacarb exposure on the pre challenge (0 h) plasma glucose levels. However, the salinity challenge significantly increased the plasma glucose levels of all treatments after 24 h. Plasma glucose of the CP and 1.5IT groups remained elevated at 48 h after the challenge. After 72 h challenge, the plasma glucose levels of the 0.75IT and 1.5IT groups, but not CP group, were significantly higher than those of the CN groups.

There was no significant difference in the pre challenge sodium values; however, saltwater exposure resulted in significant sodium elevation during 72 h (Fig. 4).

In comparison to the CN group, indoxacarb treatment had no effect on pre challenge of plasma chloride levels. Plasma chloride of the CP and 0.75IT was similar to that of CN after 24 h, but 1.5IT had higher chloride levels compared to CN. After 48 h, all treatments had higher chloride levels compared to CN; however, after 72 h, only CP and 0.75IT treatments had higher chloride levels that of the CN treatment (Fig. 5).

According to Fig. 6, plasma potassium levels did not change in response to indoxacarb treatments (pre challenge values). The plasma potassium values in all groups were significantly higher than that measured in the CN group, from 24 h after the challenge and thereafter. Also, plasma potassium in the 0.75IT and 1.5IT groups was significantly higher than the CP group after 24 h.

Fig. 7 shows that pre challenge plasma calcium levels had no change in comparison to the CN group. Compared to the CN group, salt water exposure caused a significant reduction (24 h) and increase (72 h) in the plasma calcium values of 0.75IT and 1.5IT groups.

4. Discussion

It is the first investigation about the effect of an oxadiazine pesticide, indoxacarb, on fish saltwater tolerance. Cortisol is generally used
as an indicator of stress (Barton, 2002; Bonga, 1997). It is well-established that stressors cause transient increase in plasma cortisol levels, generally to provide demanded energy to cope with stress (Aluru and Vijayan, 2009; Barton, 2002; Bonga, 1997). However, cortisol secretion is also necessary when fish exposed to saltwater, as this hormone increases energy mobilization [Hoseini and Hosseini (2010); see below], chloride cell number and size (McCormick, 1990) and Na⁺, K⁺-ATPase activity (Madsen, 1990) during saltwater challenge. In C. carpio this elevation was observed 24 and 48 h after saltwater challenge which is due to involvement of cortisol in osmoregulation and osmotic stress as mentioned above. The cortisol response in C. carpio pre-exposed to 1.5 mg/L of the indoxacarb treatment was completely inhibited and 0.75 IT group’s response occurred with a 24-h delay compared to the CP group. The blocked cortisol response shows indoxacarb interferes with the HPI axis, probably at either adrenocorticotrophic hormone (ACTH) group. The blocked cortisol response shows indoxacarb interferes with ACTH and cortisol synthesis (Benguira et al., 2002; Cericato et al., 2008 and Zhang et al., 2015). Further, the HPI impairment after exposure to agrochemicals may be due to disruption of metabolic changes in fish liver. Gravel and Vijayan (2007), Hori et al. (2008) and Zhang et al. (2015) respectively postulated that the mode of action of salicylate and phenol involves two key proteins, STAR and liver GR, which are critical for the cortisol production and target tissue responsiveness to this steroid. In is necessary to conduct further studies on indoxacarb effects on the HPI axis to understand its toxic mechanism. However, based on the current results, such a depression in cortisol secretion in response to stress is detrimental to carp, as cortisol elevation improve the fish survival in saltwater (discussed above). Early fish mortality in the 3IT group supports this hypothesis.

As mentioned above, one of the important effects of cortisol in stressful condition is energy supply characterized by increase in circulating glucose. In this study, CP group showed an expected glucose change after saltwater exposure, an early increase lasted for 48 h which recovered after 72 h. This pattern shows that fish energy expenditure increased after saltwater challenge, but the fish was capable to cope with stress after 72 h, similar to those reported previously in this species (Hegab and Hanke, 1984; Hoseini and Hosseini, 2010). However, in the indoxacarb-treated fish, glucose remained elevated until 72 h saltwater exposure, suggesting that indoxacarb exposure deteriorates carp saltwater adaptation. In this case, Hoseini and Hosseini (2010) showed that carp survived in saltwater exposure had recovered glucose levels after 72 h. Because of no difference in plasma glucose after 21 d exposure to indoxacarb, it is believed that higher glucose in the indoxacarb-treated fish is due to interference of glucose homeostasis rather that additive effect of the pesticide and saltwater exposure. Also, fluctuations in plasma glucose in 0.75 IT suggest interfered glucose homeostasis. In this case, there is only one study showing that xenobiotics exposure impaired glucose response to an acute air exposure (Nascimento et al., 2012). This might be due to interference in the function of hormones and enzymes involved in carbohydrate metabolism (Gravel and Vijayan, 2007; Hoseini et al., 2014; Nascimento et al., 2012). Seemingly, there is a need for further studies on this topic to understand underlying mechanisms.

Pesticides exposure markedly impair hydromineral balance by affecting gill and kidney structure and ion transport pumps (Cengiz, 2006; Hajirezaee et al., 2016; Hoseini et al., 2012; John, 2007; Katuli et al., 2014; Nascimento et al., 2012; Yildirim et al., 2006). However, little is known about the adverse effects of the pesticides on subsequent saltwater adaptation. Plasma ions showed no significant difference after 21 d indoxacarb exposure suggesting maintained hydromineral balance during the pesticide exposure. However, plasma sodium, potassium and chloride levels of the CP after salinity challenge are consistent with the previous investigations which have confirmed that common carp is a stenohaline fish and hyperosmoregulator in exposure to salt water to preserve water influx and prevent dehydration (Hegab and Hanke, 1984; Van der Linden et al., 1999). Slight higher plasma chloride and sodium levels were observed in the indoxacarb-treated fish after 24 h saltwater exposure suggesting indoxacarb exposure slightly impaired these ions regulation. There is only one study in this case showing that diazinon exposure of Caspian roach (Rutilus caspicus) interfered plasma sodium, chloride and potassium regulation after saltwater exposure (Katuli et al., 2014). Plasma calcium levels of indoxacarb treated groups showed mixed changes during salinity challenge. This may be due to adverse effects of indoxacarb on calcium-regulating organs such as kidney and gill [as several studies show toxics adversely affect fish gill and kidney (Cengiz, 2006; Hoseini et al., 2016a; Xing et al., 2012; Yildirim et al., 2006)] and hormones such as calcitonin [as previous studies showed that toxics interferes with calcium regulation and calcitonin function (Mishra et al., 2005; Suzuki et al., 2006)]. The only available study on Caspian roach showed that pre-exposure to diazinon resulted in plasma calcium fall in comparison to a control group after saltwater challenge (Katuli et al., 2014). Thoroughly, ionic disturbance in the indoxacarb-treated groups occurred sooner than the CP, which may be as a result of tissue damages as gill and kidney have an important role in fish osmoregulation (Perry et al., 2003) and reduction of energy reserve as hydromineral balance maintenance needs energy expenditure (Soengas et al., 2007).

5. Conclusion

In conclusion, the present results demonstrate that sub-lethal exposure to indoxacarb impairs cortisol response to saltwater stress. Also, it interferes glucose metabolism and hydromineral balance. All these effects threaten the fish life chance in response to saltwater stress.

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References

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Fig. 7. Plasma calcium levels in common carp exposed to indoxacarb and challenged with saltwater. Different letters above the bars indicate significant differences among treatment at each time (ANOVA and Tukey tests; n = 6; F = 7.74, P < 0.0001).