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Altered glycometabolism in zebrafish exposed to thifluzamide

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HIGHLIGHTS

- Thifluzamide increased liver glycogen levels in zebrafish.
- Thifluzamide reduced blood glucose levels in zebrafish.
- Aerobic glycolysis in liver was inhibited by thifluzamide.
- Anaerobic glycolysis in liver was inhibited by thifluzamide.
- Pentose phosphate pathway in liver was stimulated by thifluzamide.

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ABSTRACT

Thifluzamide exerts toxic effects to zebrafish and causes liver mitochondrial damage. To better understand the further mechanism, adult zebrafish were exposed to a range of thifluzamide concentrations (0, 0.019, 0.19, and 1.90 mg/L) for 28 days. In response to 1.90 mg/L exposure, liver glycogen significantly increased and blood glucose decreased. The expression of genes related to glycometabolism showed corresponding changes. Genes related to mtDNA replication and transcription and genes participating in mitochondrial complexes showed altered expression, which might lead to the inhibition of the tricarboxylic acid cycle (TCA). Additionally, the activity of glucose-6-phosphate dehydrogenase (G6PDH) was markedly increased at 1.90 mg/L, which might result in the activation of the pentose phosphate pathway. Moreover, the activity of lactate dehydrogenase (LDH) was significantly reduced at 1.90 mg/L, which might indicate that anaerobic glycolysis was inhibited. This study suggests that the altered gene expression and enzyme activities might be responsible for changes in glycometabolism, as evidenced by the altered expression of glycometabolism-related genes, the increased amount of glycogen in the liver and the decreased blood glucose levels. Overall, thifluzamide caused dysfunctional glycometabolism and led to events that might contribute to various thifluzamide-induced abnormalities in zebrafish.

1. Introduction

In fish, chemical and environmental stress may influence physiological and biochemical functions, such as development, growth, and reproduction (Scott and Sloman, 2004; Venkataramana et al., 2006). In addition, toxic contamination by pesticides and heavy metals can change fish serum biochemistry due to damage and dysfunction in tissues caused by the toxicants (Firat et al., 2011). Biochemical alterations are sensitive indicators that can reveal toxicity before the harmful effects are apparent in fish. The effect of a toxicant on the activity of an enzyme is one of the most important biochemical indicators of stress (De Zwaan et al., 1993; Das and Mukherjee, 2003; Prashanth and Neelgund, 2007). When tissues are diseased because of a toxicant, the activity of an enzyme may be inhibited or increased. Because some enzymes help to metabolize carbohydrates, decreases or increases in their levels may be sufficient to provide information of diagnostic value (Begum, 2009). Thus, we advocate a biochemical approach to provide an early warning of potentially hazardous changes in stressed fish. Furthermore, it is essential to elucidate the biochemical effects on zebrafish as a matter of scientific interest.

Abbreviations: dpe, days post-exposure (count from the exposure beginning).

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Evaluation of biochemical parameters in zebrafish helps to identify disturbances in metabolism.

Thifluzamide is an extremely broad-spectrum, widely used fungicide. It is effective in controlling an extensive range of basidiomycete diseases. Reports showed that thifluzamide had registered for controlling rice sheath blight in China (O'Reilly et al., 1992; Gupta and Gajbhiye, 2004). When it enters aquatic environments, more consideration should be given to the potential hazards caused by thifluzamide. Thifluzamide has been shown to exert toxic effects on adult zebrafish and produces physiological and biochemical changes in zebrafish livers. Its interference with succinic dehydrogenase (SDH), its inhibition of respiratory chain complexes, its interaction with the mitochondrial membrane, and the damage it causes to cells and organelles, such as mitochondria, are some of the reported molecular pathways by which thifluzamide exerts its toxic effects on the zebrafish liver (Yang et al., 2016). The activities of enzymes are intimately linked to metabolism. SDH embedded in the internal membrane of mitochondrion is involved in the key route of glucose metabolism, viz tricarboxylic acid cycle (TCA). Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes pentose phosphate pathway, which is active in liver. The pentose phosphate pathway is a minor one of glucose metabolism, compared with TCA (Kamalaveni et al., 2001). Lactate dehydrogenase (LDH) catalyzes anaerobic glycolysis (Ramakritinan et al., 2005). The alteration of SDH, G6PDH and LDH activities can lead to glucose metabolic disturbance and reflect toxicant stress (Kamalaveni et al., 2001; Ramakritinan et al., 2005). Many chemicals can affect glucose metabolism. For example, perfluoro octane sulfonate (PFOS) interferes with blood sugar levels in mice (Lv et al., 2013; Wan et al., 2014). Glucose metabolism of fish can be changed under toxic conditions via altering the enzymes activity (Dayananda Reddy et al., 1984).

In addition, a previous study showed that the target of thifluzamide toxicity is the liver (Yang et al., 2016). Liver is the principal organ for glycogen storage. After thifluzamide exposure to zebrafish, whether the liver toxicity can cause dysfunctional glycometabolism? In the present study we monitored changes in glycometabolism in the livers of zebrafish by exposing them to different sub-lethal concentrations of thifluzamide. Here, adult zebrafish were exposed to thifluzamide for 28 days. The levels of liver glycogen and blood glucose were tested. The expression of genes related to mtDNA replication/transcription and mitochondrial complexes and the activity of G6PDH and LDH were determined. This paper aims to provide an integrated understanding of the effects of thifluzamide on glucose metabolism in zebrafish.

2. Materials and methods

2.1. Reagents and chemicals

Standard water used for the exposure experiments contained 2 mmol/L Ca^{2+}, 0.5 mmol/L Mg^{2+}, 0.75 mmol/L Na^{+} and 0.074 mmol/L K^{+}. Thifluzamide (95%, CAS: 130000-40-7) was obtained from the Beijing Huarong Biological Hormone Plant and the stock solution used for drug exposure was prepared with acetone AR and Tween-80. Glycogen, pyruvate, lactate and LDH assay kits were purchased from Nanjing Jiancheng Bioengineering, Inc. (Nanjing, Jiangsu, China). G6PDH assay kits were purchased from the Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). All other reagents were analytical grade with the highest purity available.

2.2. Zebrafish cultivation and exposure

Adult zebrafish were maintained according to Mu et al. (2013). Zebrafish (weighing 0.20–0.40 g, 2.00–4.00 cm long) were purchased from a fish shop (Gao feng Aquarium, Beijing) and domesticated for at least 14 days in standard lab water at 27 ± 1 °C, a pH of 7.5 ± 0.5, and water hardness up to 205 ± 13 mg/L (CaCO3) with a photoperiod of 14 h:10 h (light:dark). The water was aerated and dechlorinated before being used. Zebrafish were fed twice every day with live brine shrimp (Artemia, Futian Brand, Japan).

The exposure experiment was conducted in accordance with the previous study (Yang et al., 2016). Sixty adult fish were exposed in a 60-L glass aquarium with test solutions. A series of concentrations (0, 0.019, 0.19, 1.90 mg/L) was prepared using standard water based on pre-experimental data. Each group ran in three replicates. The exposures lasted for 28 days and were renewed daily. Solvent control contained the same acetone content and Tween-80 with the highest dosage solution (0.1 mL/L acetone and 1 μL/L Tween-80). The fish were fed daily with dry food (equivalent to 2% of body weight) until 24 h prior to sacrifice. The external conditions during exposure, including humidity, temperature and light cycle, were the same as in the culture environment. At the end of exposure, the body weight was measured. The livers were excised, weighed, frozen in liquid nitrogen and stored at −80 °C for future analysis. The experiments were performed in accordance with current Chinese legislation and were approved by the independent animal ethics committee at China Agricultural University.

2.3. Hepatosomatic index and measurement of blood glucose

At the end of exposure, the body weights and liver weights of zebrafish were measured, and the hepatosomatic index (HSI) was calculated.

$$\text{HSI} = (\text{liver weight}/\text{body weight}) \times 100$$

At the end of exposure, the fasting blood glucose was determined from the fish tail by using an automatic glucose monitor (Accu-Chek Performa, Roche).

2.4. Histopathology and liver glycogen measurement

At the end of the test, 5 fish were randomly selected from the aquarium and were fixed in 10% buffered formalin for 24 h, then embedded in paraffin and sliced vertically into 4 μm thick sections with a freezing microtome (ERMAINC AO820). Finally, the sections were stained with PAS as well as a combined method using periodic acid-Schiff–stain. A light microscope (OLYMPUS BH2) was used to examine the samples. Integrated optical density (IOD) for glycogen sections was measured by the Pathological Image Analysis System 6.0.

At 28 days post-exposure (dpe), 6 zebrafish were randomly selected from each aquarium for liver sampling and glycogen measurement. Liver glycogen was measured by using assay kits (Jiancheng, Nanjing, China) and calculated according to the manufacturer's instructions.

2.5. G6PDH activity analysis

Six zebrafish were selected from each aquarium at the end of exposure for the liver sampling. G6PDH activity was determined by using assay kits (Solarbio, Beijing, China) and calculated in accordance with the manufacturer's instructions. The total protein content was determined by using a bicinchoninic acid protein assay kit (CW Biotech, Beijing, China).