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The metabolic responses of crucian carp blood to Cyprinid herpesvirus 2 infection

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ABSTRACT

In recent years, Cyprinid herpesvirus 2 (CyHV-2) has severely affected the culture of crucian carp *Carassius auratus gibelio*, causing massive economic losses. However, the responses of crucian carp during infection with CyHV-2 have not been explored. In this study, physiological and biochemical indicators and enzyme activities of crucian carp before and after infection with CyHV-2 were analyzed. The number of white blood cells in the diseased fish was significantly increased. Compared to control group, red blood cell content, hemoglobin content, mean corpuscular volume, mean corpuscular hemoglobin content and mean corpuscular hemoglobin concentration in the experimental group were significantly decreased. In the diseased fish group, the levels of the glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, alkaline phosphatase, lactate dehydrogenase and urea nitrogen in the blood were also significantly increased compared to the control group. In addition, the activity of serum total antioxidant capacity, acid phosphatase, glutathione-S transferase and catalase in diseased fish were significantly higher than in the control group. LC-Q/TOF-MS analysis platform was used in this study for the first time to detect changes in the serum metabolome of crucian carp infected with CyHV-2. A total of 79 differential metabolites have been identified. Significant increases in concentrations were found for several carbohydrates (glucose, mannose, ribose, etc.), fatty acids (pentadecanoic acid, arachidonic acid, palmitic acid, eicosapentaenoic acid, hydroxylic tetracarbonate alkenoic acid, linoleic acid and its derivatives) and most of the amino acids and their derivatives. However, guanine, cytosine, uridine and VB2 were significantly decreased. KEGG pathways enrichment showed that amino acid metabolism and lipid metabolism were severely influenced. The above results provide a theoretical basis for the infection mechanism of CyHV-2

1. Introduction

Crucian carp *Carassius auratus gibelio* is one of the important freshwater cultured fish in China. It is widely distributed, delicious and nutritious and has been deeply favored by consumers (Lu et al., 2009). In recent years, as the scale of farming continues to expand, many diseases have begun to erupt and hinder the development of the aquaculture industry. In 2012, a severe gill hemorrhagic disease occurred in farming areas, causing massive deaths and serious economic losses (Wang et al., 2016; Danek et al., 2012). The gill lamellae and filaments of the diseased fish suffered from holes, shortened length, and a large number of tissue cells fell off. Epithelial cells and gill lamellae capillaries were shed. Mucous cells and mitochondria rich cells are necrotic. The number of cells in gill was significantly reduced (Wu et al., 2013). The identified cause of the disease was shown to be Cyprinid herpesvirus 2 (CyHV-2).

CyHV-2 was first discovered in 1992 in goldfish suffering from hematopoietic organ necrosis, and the pathogenic virus of the disease was called Goldfish Hematopoietic Necrosis Virus (GFHNV) (Jung and Miyazaki, 1995). Subsequently, the disease has appeared in the United States, Australia, New Zealand, Taiwan, the United Kingdom, Hungary and other places, and thereafter the epidemic spread worldwide (Chang

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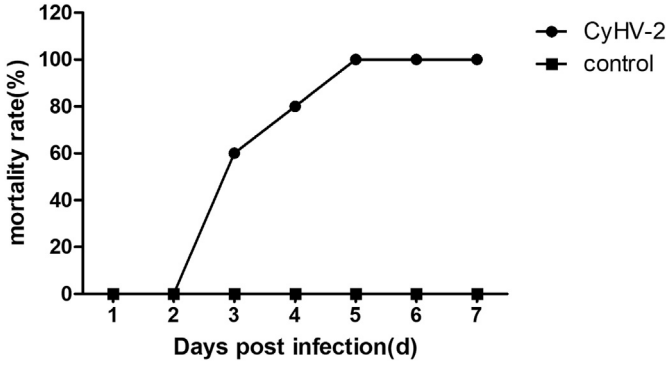

Fig. 1. Fish cumulative mortality following injection with CyHV-2 or control PBS.

et al., 1999; Goodwin et al., 2006; Stephens et al., 2004; Jeffery et al., 2007). The first case of CyHV-2 infecting crucian carp in China was reported in 2012 (Wang et al., 2012). Subsequently, CyHV-2 has been widely distributed in China. To understand the immune response of the crucian carp capable of resisting CyHV-2, a key step is to investigate the physiological changes occurring in infected fish.

Metabolomics is an interdisciplinary field that emerged in the post-genomics era. Its main goal is to quantitatively study the multiple dynamic responses of metabolite levels that are produced by living organisms in response to external stimuli, pathophysiological changes, and mutations in their genes. The technology was quickly applied by researchers to study the interaction between host and pathogen in infected flounders (Cho et al., 2017). Hematological parameters have a close relationship with the organism's metabolism, nutritional status and disease. When a fish is stimulated by external environmental factors, such as low temperature stress, low oxygen, environmental pollution and disease, the hemoglobin indicators, and physiological and biochemical indicators will change accordingly to maintain a steady physiological state as a protective response. Therefore, changes in blood composition are widely used to evaluate fish health status, nutritional status and adaptation to the environment (Bhaskar and Rao, 1990). Generally, in the case of fish disease, the tissues in the body are easily damaged, resulting in the destruction of cells, the release of enzymes, and the increase of serum enzyme activity. Therefore, serum enzyme activity not only represents the level of tissue metabolism, but is also often used as a clinical diagnostic indicator (Kraljević et al., 2008). At present, there are many published studies on blood biochemical indicators that are commonly used in vertebrate research, especially human and livestock. However, there are few reports on the effect of a virus infection on hematological parameters, serum enzyme activity, and metabolites of crucian carp.

In this study, hematological parameters, biochemical indicators and enzyme activities of diseased fish and healthy fish were determined. Different metabolites in serum after CyHV-2 infection were analyzed using a metabolome approach. These studies provide a scientific basis for further understanding of the pathogenesis of CyHV-2 infection and help to clarify the immune mechanism of crucian carp that resist CyHV-2.

2. Materials and methods

2.1. Separation and purification of the virus

The diseased fish with obvious symptoms of a hemorrhagic disease came from a diseased pond in Baoying County, Jiangsu Province, China. PCR detection of CyHV-2 was positive (Fichi et al., 2013). The diseased fish was used for the extraction of CyHV-2. After sterilization of external surfaces with 75% ethanol alcohol, the kidney and gills of diseased fish were placed in a mortar, with 5 ml PBS added, and ground for 3–5 min on ice. The slurry was poured into a 50-ml centrifuge tube and centrifuged at 4000 r/min for 10 min at room temperature. The supernatant after centrifugation was transferred to a new 50-ml centrifuge tube, and the crude virus extract was filtered through a 0.22 μm filter.

2.2. Virus infection experiment and sample extraction

100 healthy crucian carp (300–400 g in weight) with no CyHV-2 detected by PCR were obtained from a fish pond with no disease history in Baoying county of Jiangsu province, China. Crucian carp were cultured one week before the experiment in aquaculture tanks containing aerated freshwater at 26–28 °C. Healthy crucian carp were randomly collected from the aquaculture system and placed into two groups, the experimental group and the control group, with 30 animals in each group. 0.1 ml of crude virus extract and sterile PBS were injected into the peritoneal cavity of crucian carp of the experimental group and the control group, respectively. In the 7 days following the injection, the number of dead fish in each of the two groups was counted daily and the symptoms of the diseased fish were observed. Pathological examination of spleen and kidney tissue of diseased fish was conducted by transmission electron microscope (TEM). When fish showed signs of

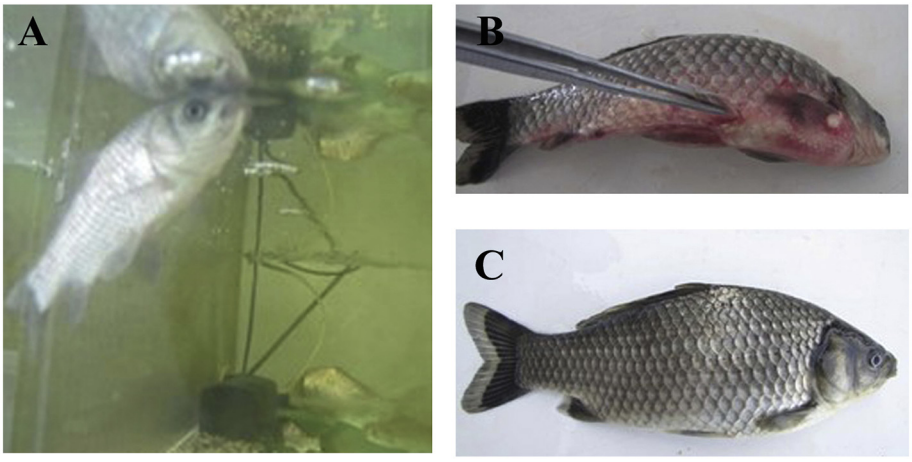

Fig. 2. Symptoms of injected fish on the 3rd day. (A) Floating head symptom, (B) operculum and abdominal subcutaneous hemorrhage symptom, (C) White tail symptom.