The Response of Antioxidant Enzyme Activity to *Streptococcus Agalactiae* Infection in Zebrafish Intestine

Hongwei Li, Yongqiong Wang, Man Zhang, Qilin Zhang*

Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500 China.

* Corresponding authors: zhangqilin88888@126.com (Q.L. Zhang)

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Abstract: Recently, *Streptococcus agalactiae* (*SA*) has become a major pathogen leading to Streptococcosis. To understand the physiological responses of zebrafish (*Danio rerio*) to *SA*, the activity of antioxidant enzymes of the intestine (24 hours post-infection, hpi, respectively) in *SA* infected zebrafish were investigated. Glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase, and peroxidase activities, were employed as biochemical indicators. GST, GSH-Px, and CAT activity, significantly changed after *SA* infection (24 hpi vs control, $p < 0.05$) in the zebrafish intestine. This study is the first exploration for antioxidant enzyme activity under pathogenic infection in zebrafish intestine. Results suggest that the *SA* infection alters the antioxidant enzyme activity in zebrafish intestine.

1. Introduction

Streptococcus agalactiae (*SA*) is a Gram-positive bacterium belonging to group B *Streptococcus*. *SA* infection can cause a variety of fish diseases, such as meningitis, sepsis, ascites and anorexia, leading to extremely high mortality rates, generally greater than 50% [1]. The intestinal mucosa plays a key role in the body's first line of defense. Zebrafish are a well-used animal for fish immunology investigations [2]. Zebrafish have been used to establish a disease model for *Salmonella*-infected fish; researchers have found that genes encoding transcription factors, immune cell surface receptors, cytokines, and chemokines presented respond acutely to *Salmonella* infection [3]. However, antioxidant enzyme activity maintains immune homeostasis and resist invasion from exogenous organisms. However, how to antioxidant enzyme activity alter between normal fish and individuals infected by *SA*.

The exploration for changes of antioxidant enzymes in fish intestine to key pathogenic bacteria is necessary to understand intestinal function in responses to bacterial infection. In this study, zebrafish were used as a model organism, and the activity of several key antioxidant enzymes were compared between *SA* treated and control fish groups. This study provides a theoretical basis for the intestinal antioxidant enzymes of fish.

2. Materials and methods

All zebrafish were treated in accordance with the recommendations from the Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Ethical Committee of Researches of Kunming University of Science and Technology. Experimental zebrafish were acclimatized for approximately five days to empty their digestive system contents using filtered freshwater, prior to their infection with *SA* strain. Samples were treated based on our previous methods.

Intestinal tissue was weighed and ground in pre-cooled homogenate medium (0.01 M Tris-HCl, 0.001 M $EDTANa_2$, 0.01 M sucrose, pH7.4), centrifuged at 3,000 r/min, and the aqueous supernatant was collected for the determination of enzyme activity [4]. The aqueous supernatant was then incubated with the enzyme substrate and read using an UV-8000ST spectrophotometer (Shanghai METASH Instruments Co. Ltd, China) at wavelengths specified. Enzyme activities were

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tested in triplicate. Glutathione-S-transferase (GST, E.C. 2.5.1.18), glutathione peroxidase (GSH-Px, E.C. 1.11.1.9), catalase (CAT, E.C. 1.11.1.6), superoxide dismutase (SOD, E.C.1.15.1.1), and peroxidase (POD, E.C. 1.11.1.7) activities were measured and used as biochemical indicators. Commercial testing kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China, http://elder.njjcbio.com/index_en.php), and all enzymatic activities were measured using kits according to the manufacturer's instructions.

3. Results and discussion

GST, GSH-Px, and CAT activities were significantly different between control and 24 hpi *SA*-treated groups ($p < 0.05$) (Table 1), while SOD and POD activities were not significantly different. In particular, the GST activity in the 24 hpi *SA*-treated group (157.92 ± 11.14) was significantly greater than the control group (72.47 ± 8.34 U/g prot); GSH-Px activity is likely to be induced after *SA* infection in the zebrafish intestine (control 44.92 ± 6.36, treatment 17.58 ± 3.47 U/g prot); CAT activity values showed a significant increase from 20.35 ± 4.11 in control to 42.67 ± 5.82 U/g prot in the 24 hpi treatment group, meanwhile.

Oxidative stress is induced by bacterial infection because reactive inflammation and immune responses greatly promote the production of reactive oxygen species (ROS) and H_2O_2 [5]. ROS and H_2O_2 levels are controlled by the collaborative action of antioxidant defense mechanisms, activities of antioxidant enzymes are potential indicators for oxidative stress, the degree of cell membrane and DNA damage, and the elimination of the excessive ROS and H_2O_2 [4]. In this study, GST and GSH-Px activities in the zebrafish intestine were found to be significantly different between control and the SA-injected group at 24 hpi. GSH-PX has a similar role to CAT in tissues with a low content of CAT, and can remove H2O2 to prevent the tissue damage caused by H2O2 [6] In addition, GST exists in a variety of tissues and functions in detoxification and peroxide removal, with GSH-PX activity and capacity for lipid peroxide scavenging [4]. Much research has been conducted on the antioxidant defensive roles of GSH-PX and GST in fish that have been subjected to environmental pollutant stress. For example, Zhou et al. found that Cu2 +was linked to increased GSH-PX activity in the tissues of silver pomfret (Pampus gentareus) [7]. Liu et al. detected significantly greater GST activity in zebrafish intestines exposed to different concentrations of zinc oxide nanoparticles (ZnO-NPs) after 4 and 24 hours, which indicated that GST could reduce the oxidative damage caused by ZnO-NPs [4]. However, little research has been designed to explore the effect of bacterial infection on these key antioxidant enzymes. Here, our results indicate that the lower GSH-PX activity in SA-treated intestines may be due to the functional impairment of the antioxidant system in the intestine, which shows that bacterial exposure could promote damage of the zebrafish intestine, as reported for zebrafish that were exposed to ZnO-NPs [4]. In this study, SA stress increased GST activity, suggesting that SA infection can induce the oxidative stress response in the zebrafish intestine to decrease the oxidative damage caused by the immune system and inflammation.

Table 1 Effect of *Streptococcus agalactiae* infection on the activity of antioxidant enzymes (GST, GSH-Px, CAT, SOD, POD) in the zebrafish intestine. Different superscripts identify significant differences.

	Control group	*SA*-infected group (24 hpi)
GST (U/g prot)	72.47 ± 8.34^{a}	157.92 ± 11.14^{b}
GSH-Px (U/g prot)	44.92 ± 6.36^{a}	17.58 ± 3.47^{b}
CAT (U/g prot)	20.35 ± 4.11^{a}	42.67 ± 5.82^{b}
SOD (U/g prot)	0.73 ± 0.12	0.91 ± 0.27
POD (U/g prot)	0.27 ± 0.08	0.34 ± 0.15

CAT provides the first line of defense to clean up ROS [8]. Greater CAT activity was observed in zebrafish intestines infected by *SA* at 24 hpi compared to the control group, suggesting that bacterial infection affects the sensitivity of antioxidant enzyme. Therefore, CAT may be a potential molecular

immune indicator that can be used as an early indicator for fish *SA* disease. In the present study, the antioxidant SOD and POD activities were not significantly different between control and 24 hpi treatment groups, indicating that *SA* infection inhibited part of the antioxidant enzyme system. Under these conditions, free radicals may not be effectively scavenged, resulting in a loss of the balance between free radical production and scavenging, and loss of immune defensive functions, as reported in previous studies [9].

4. Summary

The activity of GST, GSH-Px, and CAT in the zebrafish intestine was affected by *SA* infection. This study gives novel insight into the effect of *SA* on intestinal microbiota composition and diversity in zebrafish, and provides valuable information for understanding the immune response from an enzymatic perspective within the zebrafish intestine.

Acknowledgments

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