Effect of Cefotaxime on the CAT Activities and GSH Contents of Zebrafish

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Abstract. In order to define eco-toxicity effect of cefotaxime on zebrafish, the indoor exposure method was used to study the impact of cefotaxime on zebrafish. In this study, zebrafish was exposed to cefotaxime of 1mg/L, 5mg/L, 25mg/L and 125mg/L for 15 days to study the effect of Catalase (CAT) activities and Glutathione (GSH) contents. According to the experimental data, the CAT activities and GSH contents in zebrafish muscle tissue had changed significantly during the period of exposure. The experimental results show that the activities of CAT in four concentration groups were significantly inhibited (P<0.01). The CAT activities in the 1 mg/L and 5 mg/L groups showed the "Λ" type change, inhibited first and induced later. But in the 25 mg/L and 125 mg/L groups, the CAT activities were inhibited all the time. Cefotaxime had a significant effect on GSH content in the muscle tissue of the zebrafish at the early stage of exposure, rapidly increase to the maximum at the early stage and rapidly decrease to the minimum on the 6th day. During 6th day to 15th day, the contents of GSH in the zebrafish were basically stable at the level of control. The experimental results show that the CAT activities and GSH contents in zebrafish muscle tissue had changed significantly.

1. Introduction

China is one of the largest producers of antibiotics in the world. In 2011, China's production of chemical raw materials has reached 296.01 million tons[1]. In addition to the use of antibiotics for the treatment of bacterial and human infectious diseases in humans and animals, antibiotics are also widely used as intensifiers and feed additives in intensive livestock husbandry and aquaculture[2]. Due to its low metabolic rate, most of the antibiotics are excreted into the environment in the form of original drugs or metabolites, resulting in the increase of antibiotic residues in environmental media such as water and soil[3].

Cephalosporin has broad spectrum antimicrobia and strongly bactericidal ability. It is the largest clinical application of a class of anti-infective drugs all over the world. Due to the large demand, the production of cephalosporin has been increasing year by year[4]. The domestic cephalosporin antibiotics industry mainly focuses on the production of low value-added raw materials and intermediates. The production process has a large amount of raw materials, low yield and large amount of waste. In the production chain, it mainly involves the production of basic raw materials, cephalosporin antibiotics intermediates, cephalosporin antibiotics and pharmaceutical preparations[5]. Antibiotic production will inevitably produce a large number of high concentrations of pollutants in...
the wastewater. The wastewater contain a large amount of refractory and biologically toxic substances, which pollute the surrounding water and threaten the aquatic organisms in the receiving water\textsuperscript{[6]}. At present, most of the research about cephalosporin antibiotics focuses on the treatment of cephalosporin antibiotic wastewater, few studies on its own ecotoxicity.

Zebrafish have become standardized organisms recommended by the International Organization for Standardization because they are sensitive to environmental changes\textsuperscript{[7]}. Hydrogen peroxidase, also known as Catalase (CAT), is a type of terminal oxidase widely found in animals, plants and microorganisms. It is a biological defense system established during the biological evolution Key enzyme\textsuperscript{[8]}. Glutathione (GSH) is an important water-soluble antioxidant in animal body, low molecular weight scavenger, GSH content is an important factor in measuring the body's antioxidant capacity. CAT and glutathione peroxidase (GSH-Px) together eliminate H$_2$O$_2$ produced by superoxide dismutase disruption of O$_2^-$ in vivo\textsuperscript{[9]}. When H$_2$O$_2$ concentration is low, GSH-Px catalyze the organization of peroxide, when the H$_2$O$_2$ concentration is higher, CAT play the role\textsuperscript{[10-11]}

In this study, cefotaxime used as the research object, its determination in cell and gene level on zebrafish muscle tissue CAT and GSH effect, determine the ecological effects of cefotaxime sodium on aquatic organisms, and provide the scientific basis for ecological risk assessment.

2. Materials and Methods

2.1. Experimental Material

2.1.1. Experimental Equipment and Instrument UV-visible light spectrophotometer (UV-2550); analytical balance (EL204); Pipette (Pipet-Lite; TopPette Pipettor); whirlpool mixer (XW-80A); high speed centrifuge (TG16-WS); water bath kettle (DK-S26); hypothermia refrigerator (BBC-226STV); 5L glass aquarium; aeration device; heating rod; thermometer; measuring cylinder; manual glass homogenizer.

The kit of CAT and GSH were purchased from Nanjing biological engineering research institute.

2.1.2. Test Organisms Zebrafish were obtained from Shijiazhuang Huaita aquafarm. Mean length of zebrafish used was 30±3mm. Mean weight of zebrafish was 0.25g±0.05g. Stop feeding before entering the laboratory, put the fish in the 5% salt water to disinfection. Feeding the fish with fully aerated dechlorinated tap water in the laboratory for 7d. Experimental water in line with "Fishery water quality standards (GB11607-1989)".

2.2. Experimental Method

The subacute toxicity experiment set up according to four concentration groups and a control group. The experiment period was 15 days. The experimental concentrations of the four groups were set as 1, 5, 25 and 125 mg/L, respectively. Each concentration group had 3 parallel groups. Add the experimental liquid to 5 L glass fish tank, put 25 zebrafish into every fish tank at random, measured zebrafish muscle tissue CAT, GSH indicators once every 2 days. During the experiment, change the experimental solution every day. Keep the pH of the experimentation solution at 7-8, the water temperature at 22-24°C.

2.3. Assay Method

Take three zebrafish from the exposure concentration group, dissect them, then take the muscle tissue 0.2g. Rinsed in normal saline and placed in a glass homogenizer. Pipette was used to add 9 times volume of saline, ice water bath conditions homogenization, tumble the rod dozens of times (about 7min), fully ground. The prepared 10% tissue homogenate was centrifuged at 3000 r/min for 10 min using a conventional centrifuge. A part of the supernatant was diluted with 0.9% physiological saline to form a 1% tissue sample, the remaining supernatant was stored at 4°C. Activities of CAT and GSH
content in samples were estimated by the method of CAT test kit (UV spectrophotometry) and GSH test kit.

2.4. Statistical Analysis
Statistical results are expressed as mean ± SD of three sets of parallel data. The experimental data were analyzed by one-way ANOVA using SPSS17.0 statistical software. Significant difference analysis was made between the two groups on the same day by the least significant difference (LSD) method. 0.01<P<0.05 means significant difference; P<0.01 indicates that the difference is significant.

3. Results and Discussions

3.1. Effect of cefotaxime on the CAT activities in muscle of zebrafish
Effect of cefotaxime on the CAT activities in zebrafish muscle tissue was shown in table 1.

Table 1. Effect of cefotaxime on the CAT activities in zebrafish muscle tissue

| Concentration (mg/L) | 3d          | 6d          | 9d          | 12d         | 15d         |
|----------------------|-------------|-------------|-------------|-------------|-------------|
| 0                    | 22.47±1.674 | 20.47±0.973 | 19.79±1.988 | 22.64±1.820 | 22.83±2.681 |
| 1                    | 15.94±1.018**| 21.49±1.545 | 23.83±0.938**| 30.87±2.366**| 18.57±0.031* |
| 5                    | 9.75±0.005** | 18.14±1.755* | 24.47±0.978** | 33.69±0.273** | 32.14±0.036** |
| 25                   | 15.80±0.424** | 19.44±0.451 | 17.25±2.915 | 19.36±0.304* | 22.71±0.200 |
| 125                  | 16.56±0.834** | 16.76±0.131** | 21.73±0.890 | 12.89±1.020** | 18.58±0.210* |

The same day all the data are compared with control group, *p<0.05, **p<0.01.

As shown in table 1, cefotaxime led pressure to the body's antioxidant system of zebrafish. CAT's main role is to catalyze the H$_2$O$_2$ to H$_2$O and O$_2$, so that cells to avoid the poisoning of H$_2$O$_2$\[^{12}\]. On the 3rd day, The activities of CAT in four concentration groups were significantly inhibited (P<0.01) compared with the control group, CAT activity was inhibited by reactive oxygen species (ROS). Except for the maximum concentration (125mg/L), the activities of CAT in the other three concentration groups was increasing, the reactive oxygen species were eliminated and the inhibition of CAT was weakened on the 3rd day to the 6th day. In the group of 1mg/L and 25mg/L, the activity of CAT restore the control level (P>0.05) on the 6th day, H$_2$O$_2$ was mainly catalyzed by GSH-Px. On the 9th day, H$_2$O$_2$ accumulated to a certain concentration, CAT activity was induced to increase (P<0.01) and began to catalyze H$_2$O$_2$ reaction. On the 12th day, the CAT activity in the 1mg/L and 5mg/L groups was more intense than that in the 9th day (P<0.01), at this time, the two concentration groups reached the maximum of the respective concentration group, this shows that H$_2$O$_2$ is still maintained at a high level, they need to induce more CAT enzyme involved in its clearance reaction. With the consumption of CAT, the activity of CAT began to decline in the two concentration groups. The activity of CAT in the group of 1mg/L dropped sharply, lower than that in the control group on the 15th day (0.01< P<0.05). But the activity of CAT in the group of 5mg/L still maintained the high level (P<0.01). The 25mg/L exposed groups always lower than the control group from 3rd to 9th days. The maximum concentration group (125mg/L) was inhibited all the time except the 9th day(0.01< P<0.05). It is consistent with the experimental results of the effect of tetracycline on CAT in zebrafish, in this experiment, Liang et al. find high concentration of tetracycline significantly effect the activity of CAT\[^{13}\]. Probably due to the high concentration of cefotaxime, excess reactive oxygen species inhibited the activity of CAT.

In summary, cefotaxime has a significant effect on the CAT activity of zebrafish. The CAT activities in the 1mg/L and 5mg/L groups showed the "A" type change, inhibited first and induced later. On the 12th day, reaching the maximum activity. The CAT activities in the 25mg/L and 125mg/L groups were inhibited all the time. High concentration of cefotaxime inhibited the activity of CAT obviously.
3. 2. Effect of cefotaxime on the GSH contents in muscle of zebrafish

Effect of cefotaxime on the GSH contents in zebrafish muscle tissue was shown in table 2.

| Concentration (mg/L) | GSH contents (mgGSH/gprot) 3d | 6d | 9d | 12d | 15d |
|----------------------|---------------------------------|----|----|-----|-----|
| 0                    | 1.306±0.031                     | 1.672±0.303 | 1.839±0.236 | 1.727±0.228 |
| 1                    | 2.554±0.157**                   | 0.742±0.031** | 1.544±0.434 | 1.924±0.215 |
| 5                    | 2.674±0.066**                   | 1.212±0.185 | 1.444±0.133 | 2.556±0.166* |
| 25                   | 2.877±0.009**                   | 1.306±0.031 | 1.138±0.207 | 2.267±0.350 |
| 125                  | 3.587±0.082**                   | 1.040±0.109** | 2.079±0.106 | 2.100±0.081 |

The table 2 shows there was a significant "concentration-effect" relationship between the content of GSH and the exposure concentration of cefotaxime. GSH contents in the four concentration groups at the initial exposure were significantly higher than that in the control group (P<0.01), reached the maximum GSH content of each exposure concentration: 2.554±0.157 mgGSH/gprot (1mg/L) < 2.674±0.066 mgGSH/gprot (5mg/L) < 2.877±0.009 mgGSH/gprot (25mg/L) < 3.587±0.082 mgGSH/gprot (125mg/L). Zebrafish exposed to cefotaxime for 3 days resulted in the accumulation of reactive oxygen species in the body, allowing the body to induce more non-protein antioxidant enzymes GSH to eliminate these free radicals and reduce the body's oxidative stress. With the increase of exposure time, the elimination of oxygen free radicals in the body consumed a large amount of GSH on the 6th day, and the GSH contents in the four concentrations decreased significantly compared with those on the 3rd day. Sun et al. reported that after 120 hour post fertilization perfluoroctane sulphonate infecting, the contents of GSH in zebrafish larvae decreased from (7.32±0.79) nmol/mg protein in the control group to (4.53±0.85) nmol/mg protein[14]. This result is consistent with this experimental results. GSH contents in the three concentration groups (1 mg/L, 5 mg/L and 125 mg/L) reached the minimum concentration within the exposure period on the 6th day and the group of 25 mg/L reached the minimum concentration on the 9th day. On the 9th day, the concentration of each group returned to the control level, indicating that consumption of a large GSH to eliminate the body's oxygen free radicals, the body's oxidative stress eased, GSH content gradually returned to normal. During the exposed from the 12th day to the 15th day, GSH contents in the four concentration groups were decreasing to control group level. It is indicated that after GSH was consumed in large quantities, the body's oxidative balance was basically stable. Cefotaxime did not cause irreversible oxidative damage to zebrafish muscle tissue. Cefotaxime is different from the nano-ZnO, Tian et al. find that nano-ZnO exposed led to GSH contents decreasing, zebrafish cells in the emergence of oxidative stress[15].

The GSH contents in all four groups showed a trend of "sharp decline-increase-decline". Cefotaxime had a significant effect on GSH contents in the muscle tissue of the zebrafish at the early stage of exposure, rapidly increase to the maximum at the early stage and rapidly decrease to the minimum on the 6th day. Later, the contents of GSH in the zebrafish were basically stable at the level of control. The body's antioxidant defense system restored the body's oxidative balance within a short time.

4. Conclusion

Through the subacute toxicity test, this study shows effect of different concentrations of cefotaxime on the CAT activities and GSH contents in zebrafish muscle tissue. The changes of CAT activities in 1 mg/L and 5 mg/L concentration groups were inhibited first, and reached the maximum on the 12th day. The groups of 25 mg/L and 125 mg/L were always inhibited, showed that high concentration of cefotaxime mainly inhibited the activity of CAT. GSH contents showed a trend of "sharp decline-increase-decline"; exposed during 3rd day to 6th day had a significant effect on GSH contents,
rapidly rising to the maximum on the 3rd day and rapidly decreased to the minimum on the 6th day; GSH contents returned to normal during 6th day to 15th day. According to the experimental data, the CAT activities and GSH contents in zebrafish muscle tissue had changed significantly during the period of exposure, high concentration of cefotaxime inhibited the activity of CAT obviously, but cefotaxime did not cause irreversible oxidative damage to zebrafish muscle tissue.

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References
[1] National Bureau of Statistics of the People's Republic of China. Statistical Yearbook of China Industrial Economy - 2012. Beijing: China Statistics Press, 2012.
[2] Yu Shen, Wang Min, Hong Youwei. Effect of antibiotics in the environmental medium and its microbial ecological effects [J]. Asian Journal of Ecotoxicolog, 2011, 31 (15): pp4437-4446.
[3] Xu Yonggang, Yu Wantai, Ma Qiang, Zhou Hua, Jiang Chunming. Research progress on antibiotics and their ecotoxicological effects in the environment [J]. Asian Journal of Ecotoxicolog, 2015, 10 (03): pp11-27.
[4] Xue Yu, Chen Yuying. Recent research progress of cephalosporins antibiotics [J]. Chinese Journal of Antibiotics, 2011, 36 (02): pp86-92.
[5] Zheng Wei, Chen Lvjun, Li Ying. Cephalosporin production wastewater pollution and treatment status [J]. Environmental Protection of Chemical Industry, 2009, 29 (04): pp317-321.
[6] Yu Xin. The biological toxicity of antibiotic wastewater and microbial resistance and its control technology [D]. Tsinghua University, 2014.
[7] Aragues R, Medina E T, Martinez-Cob A, et al. Effects of deficitirrigation strategies on soil salinization and sodification in a semiarid drip-irrigated peach orchard[J]. Agricultural Water Management, 2014, 142: pp1-9.
[8] Liu Lingzhi, Zhong Guangrong, Xiong Lin, Chang Yanhong, Xiao Baoqing, Luo Hui. Research and Application of Catalase [J]. Chemistry & Bioengineering, 2009, 26(03): pp15-18.
[9] N. UNER, E. ORUC, et al. Effects of Cypermethrin on Antioxidant Enzyme Activities an Lipid Peroxidation in Liver an Kidney of The Freshwater Fish, Oreochromis Niloticus and Cyprinus carpio(L). Bull Environmental Contam Toxicol, 2001, 67(5): pp657-664.
[10] Wang Yuming, Zhu Xiangwei, Ma Yong-peng, Liu Shushen, Liu Yan. Effects of Low Concentration Pentachlorophenol on SOD Activity, GSH and HSP70 Content in Ragged Carassius auratus [J]. Asian Journal of Ecotoxicolog, 2009, 4 (03): pp415-421.
[11] Peng Changcao, Sun Zhonghai. Changes in SOD and CAT Activity of Citrus Protoplasts during Cold Acclimation [J]. Journal of Huazhong Agricultural University, 2000, (04): pp384-387.
[12] Yang Jiadong, Wei Fengju, Pan Xinmin, Lv Shuping, Tang Sijing, Zhang Yingjie. Research progress of animal catalase (CAT) [J]. Heilongjiang animal husbandry veterinary, 2016, (13): pp59-62.
[13] Liang Weifang, Zhao Jianguo, Liu Xiande. Tetracycline on Zebrafish embryonic development and CAT and SOD activity [J]. Tropical Agricultural Engineering, 2017, 41 (01): pp17-20.
[14] Sun Shibo, Li Wu, Pan Xiao yuan, Lin Xin, He Qingzhi, Zeng Huaicai. PFOS impact on Zebrafish embryonic development and SOD, MDA and GSH content [J]. Practical Preventive Medicine, 2015, 22 (06): pp648-651.
[15] Tian Wenjing. Preliminary study on oxidative oxidative stress mechanism of Zinc oxide nanoparticles to Zebrafish embryos [D]. Qingdao University of Science and Technology,
2010.