

Effects of exposure to nonylphenol on expression of HSP90a of zebrafish

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Abstract

With β -actin as the reference gene by real-time quantitative polymerase chain reaction (PCR) technique to detect HSP90a gene expression by the different exposure mode (direct exposure and food exposure) at different times (0,8,16,24,32d) at different NP concentrations (2 μ g/l, 20 μ g/l) expression levels in liver, brain and muscle of the zebrafish. The results showed that 2 μ g/l concentration group NP-induced HSP90a gene expression higher than 20 μ g/l concentration group NP-induced gene expression. when the expression of HSP90a gene in liver and brain peaked at 8d, it did at 24d in muscle. The HSP90a gene relative expression of food pollution exposure treatment was higher than the relative expression of the HSP90a gene by way of directly expose zebrafish experiment treated with NP, this phenomenon maybe related to NP accumulation amount in zebrafish, the analysis result showed that HSP90a gene was sensitive to NP, it can be used as an early warning biomarker for pollutant.

Keywords

Nonylphenol, exposure, zebrafish, HSP90a, expression, effects.

1. Introduction

Nonylphenol (NP) is an important chemical raw materials and metabolic intermediate, which is the widely applied to Industrial and agricultural production, such as surfactant, antioxidants, Pesticide emulsifier, Resin modifier^[1, 2]. NP has estrogenic effects^[3], is considered the most representative of the environmental endocrine disruptors^[4]. Because NP has the characteristic of resistant to degradation and Bioaccumulation, through natural decomposition and biodegradation way to enter the ecological environment and then enter into the living body through the food chain, transfer to the higher trophic levels, leading to a series of physical and chemical reactions for the surrounding medium^[5]. low NP concentrations can have deleterious effects on the organism^[6], therefore there is the need that before NP produce the significant harm for the animal, by detect physiological and biochemical changes of the organism to warn and forecast the situation of NP pollution, it is very meaningful for the comprehensive assessment of NP on the safety of aquatic food, the risk of water ecosystem and provide the scientific basis for the development of ecological restoration program^[7].

Due to the aquatic animal zebrafish has the advantage of the strong reproductive ability, mass culture easily and high sensitivity, and approximately high homology 87% of human genes^[8, 9], so it is used as the model organism of scientific research to monitor water quality^[10]. Studies have shown that HSP90a gene has wide application, is considered to be an early potential water environment integrated pollution warning molecules^[11]. heat shock protein molecule with a wide range of different pollutants reactivity is considered a potential comprehensive early warning^[12, 13]. NP pollution can affect the growth and development of zebrafish and through the food chain leading to endocrine disrupting effects for human being^[14, 15]. Therefore, the research for HSP90a gene of zebrafish response mechanism treated with NP pollution can lay the foundation for internal mechanism of clarify the HSP90a of zebrafish as the early pollution warning molecular.

2. Materials and methods

2.1 Material

Experimental zebrafish was purchased from the flower bird fish market of Guangzhou city, body length is 3.0 ± 0.1 g, weight is 0.58 ± 0.01 cm, and the size of sample fish is three month years old. Zebrafish is cultivated in artificial glassmaking aquarium, installed pump device electric filter and changed water everyday, the water temperature is maintained at $20 \sim 23^\circ\text{C}$, fed the bloodworms at breed period.

2.2 Reagents

NP was purchased from ANPEL of shanghai company, Trizol reagent was purchased from Invitrogen, Real-time fluorescence quantitative PCR reagent was purchased from TOYOBO, Reverse transcriptase reagent was purchased from Promega, rTaq DNA Polymerase was purchased from TaKaRa.

2.3 methods

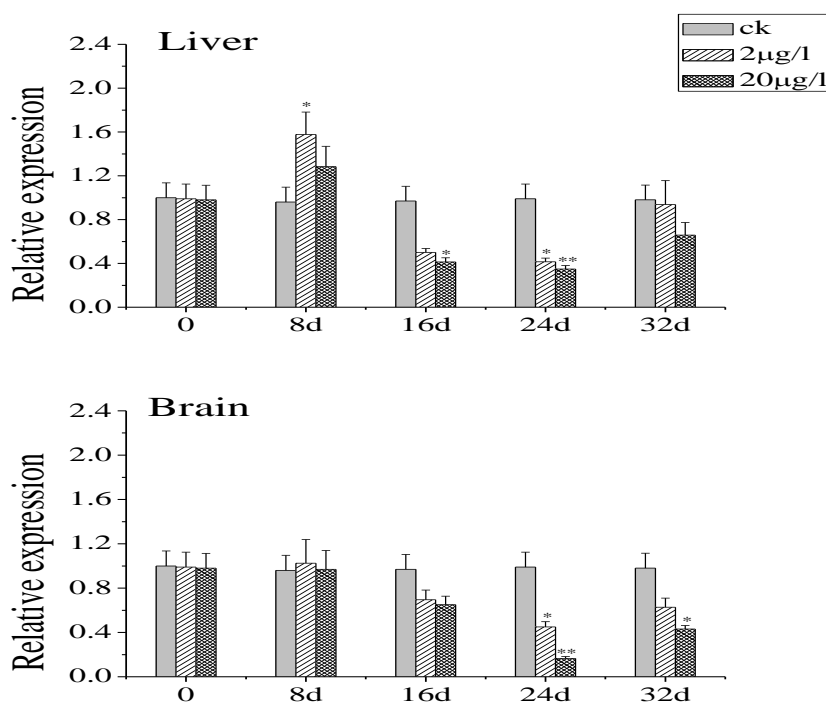
The similar size zebrafish (45 article) were randomly divided into three groups, placed in a 20L aquarium respectively, added water containing NP concentrations ($0\mu\text{g/l}$, $2\mu\text{g/l}$, $20\mu\text{g/l}$) and changed a half of water everyday. caught two zebrafish randomly to dissect brain, liver and muscle frozed in liquid nitrogen tank at continuous exposure time 0d, 8d, 16d, 24d, 32d, respectively.

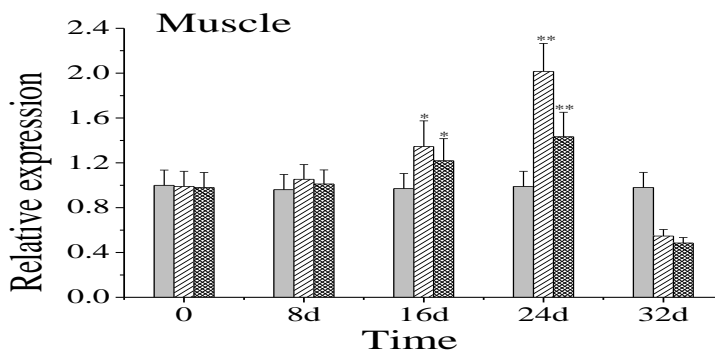
2.4 statistics analysis

The data was expressed as mean \pm S.D. The software of Origin8.0 and SPSS19.0 were used for analyzing the data; groups were compared by the LSD test, the significance test level is $P < 0.05$ or $P < 0.01$.

3. Results

3.1 Expression of HSP90a gene in three tissues of zebrafish direct exposure treated with NP





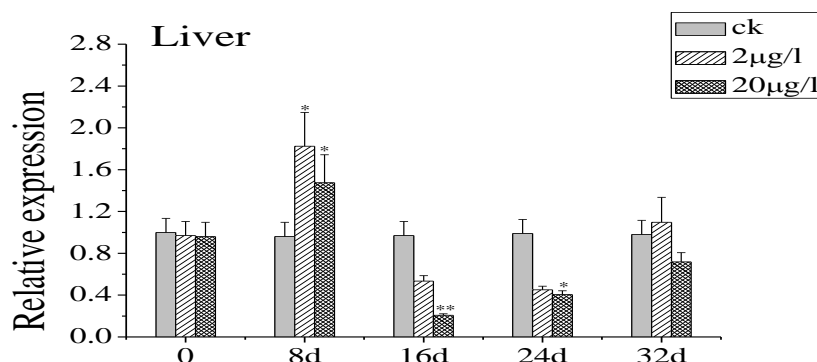
Mean ± S.D, n=3. * indicated HSP90a gene of zebrafish expression relative to the control group had significant difference treated with different concentrations of NP (P <0.05), ** indicates extremely significant difference (P <0.01) at the same time of the same figure.

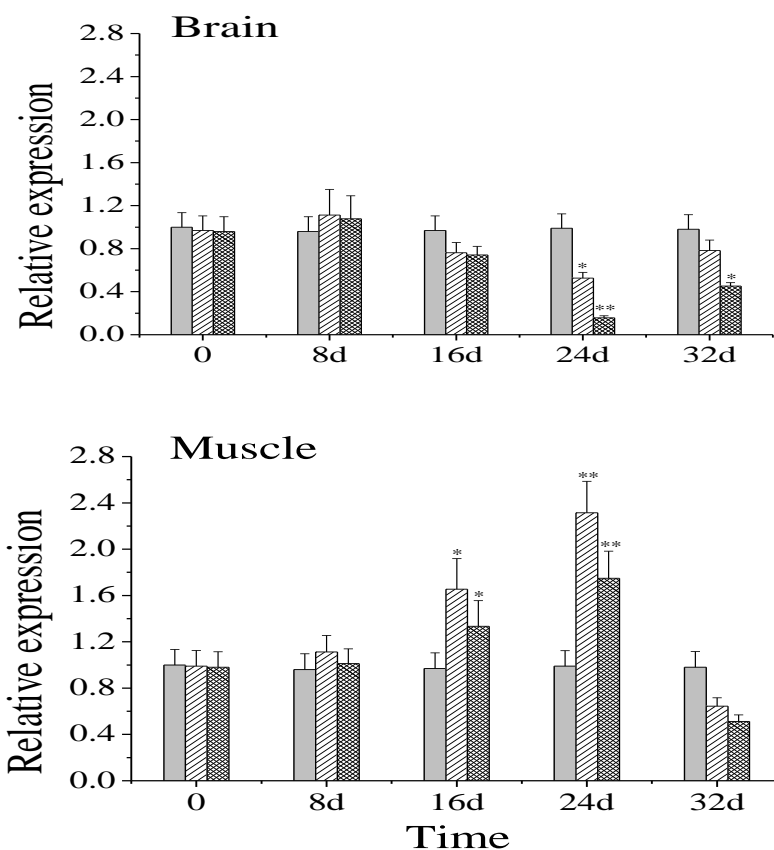
Fig.3.1 mRNA Expression of HSP90a in liver, brain and muscle of Zebrafish relative to concentration of nonylphenol (NP) in treatment

After zebrafish exposed by NP direct treatment, the expression of HSP90a genes has the specificity of the tissue. The expression of HSP90a gene of liver, brain, muscle is consistent, within a certain time frame, increased to the maximum and then decreased, with the continued extension of the processing time, the expression of gene gradually restored, the phenomenon is called time-dependent reaction^[16]. This phenomenon indicated that the molecular damage in this kind of reaction is irreversible damage, not serious and irreversible damage to the zebrafish body, But the reaction intensity and duration of the time were some differences, which is typical Stress Reactions of tissue; The expression of HSP90a gene is highly expressed in liver and brain peaked at 8d(P <0.01), the muscle appears in the 24d; 2µg/l NP concentrations induced HSP90a gene expression levels higher than 20µg/l NP concentrations in general.

3.2 Expression of HSP90a gene in three tissues of zebrafish by food exposure treated with nonylphenol

The zebrafish fed the pollution food (Daphnia magna exposed by NP), the expression law of HSP90a gene in three tissues (liver, brain, muscle) and the expression of NP directly exposed zebrafish are consistent substantially, have shown a certain degree of organization and time difference. The zebrafish fed the pollution food (Daphnia magna exposed by NP), the relative expression level of HSP90a gene in three tissues (liver, brain, muscle) were higher than the expression of direct exposed with NP, we concluded that this phenomenon may be related to the difference of accumulation amount of NP in zebrafish, the above phenomenon and the present experiment result that low NP concentrations induced the expression of HSP90a gene higher than the expression of HSP90a gene induced by high NP concentrations are coincide, that is to say, within a certain range of concentration and time, the higher amount of NP enriched in the zebrafish body, the lower expression of HSP90a gene.





Mean \pm S.D, n=3. * indicated HSP90a gene of zebrafish expression relative to the control group had significant difference treated with different concentrations of NP ($P < 0.05$), ** indicates extremely significant difference ($P < 0.01$) at the same time of the same figure.

Fig.3.2 mRNA Expression of HSP90a in liver, brain and muscle of Zebrafish relative to concentration of nonylphenol (NP) in treatment

4. conclusion

Experimental analysis results showed that NP exposure directly induced HSP90a gene specific expression in zebrafish, the expression discipline of HSP90a gene in three tissue (liver, brain and muscle) were consistent, within a certain time range, had shown to increase to a maximum value and then decreased, with the continued extension of the processing time, recovered to comparison level gradually; when the expression of HSP90a gene in liver and brain peaked at 8d, it did at 24d in muscle. The expression discipline by way of the food chain indirectly expose experiment treated with NP were consistent with the relative expression of the heat shock proteins gene by way of directly expose zebrafish experiment treated with NP were consistent with the condition of NP direct exposure to zebrafish. The relative expression was higher than the relative expression of the heat shock proteins gene by way of directly expose zebrafish experiment treated with NP, we inferred that this phenomenon may be related to different content of NP accumulation in zebrafish, which is consistent to the result that the low concentration NP induced heat shock proteins expression was higher than the heat shock proteins expression induced by the high concentration of NP. With β -actin as the reference gene to establish the detection method of the real-time fluorescence quantitative PCR technology for HSP90a gene. The analysis result showed that the HSP90a gene was sensitive to NP, it can be used as an early warning biomarker for pollutant.

Acknowledgements

The Key Project of NSFC-Guangdong Joint Fund: U1133003, National Natural Science Foundation of China: 41176104, National Natural Science Foundation of China: 41476099.

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