Comparative Toxicity of Different Crude Oils on the Cardiac Function of Marine Medaka (*Oryzias melastigma*) Embryo

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Abstract: *The acute toxic effect of different crude oils (heavy crude oil and bonny light crude oil) on embryos of marine medaka Oryzias melastigma was measured and evaluated by exposure to the water-accommodated fraction (WAF) in the present study. The cardiac function of medaka embryos was used as target organ of ecotoxicological effect induced by oil exposure. Results showed that the developing marine medaka heart was a sensitive target organ to crude oil exposure the heavy crude oil WAF was more toxic to cardiac function of medaka embryos than bonny light cured oil one. Cardiac function of medaka embryos was clearly affected by exposure to heavy crude oil WAF after 24 hours exposure and showed a dose-dependent slowing of heart rate. Furthermore, swelled and enlarged heart morphology, lowered blood circulation and accumulation of blood cells around the heart area were found. However, the toxic effect of bonny light crude oil on cardiac function of medaka embryos was comparatively low. Statistical results showed that the cardiac function was only affected by highest bonny light crude oil WAF (9.8 mg/L) exposure treatment. These findings indicated that cardiac function of marine medaka embryo was a good toxicity model for oil pollution and could be used to compare and evaluate the toxicity of different crude oils. The heart rate was an appropriate endpoint in the acute toxicity test.*

Keywords: *Marine medaka, Cardiac function, Oil pollution, WAF, Heart rate.*

Introduction

Oil is one of the most important pollutants in the marginal seas off which mainly from industrial emissions, offshore oil exploitations and oil spill incidents. With rapid industrialization development and energy demand around the world, marine environment damage caused by oil pollution has become an obvious and severe problem in coastal areas. Oil spills can result in both immediate and long-term marine ecological environmental damage like the Deepwater Horizon oil spill disaster in the Gulf of Mexico and Dalian "7.16" oil spill explosion that occurred in 2010 [3, 5, 7]. The increasing coastal oil pollutions have posed ecological risks to marine ecosystem. Some of the environmental damage caused by an oil spill can last for decades after the spill occurs. Oil pollution of coastal marine ecosystems poses a serious threat to aquatic organisms and ultimately the entire marine biological diversity.

The toxic effect of crude oil on fish embryos depends on the chemical constituents of the test oil. Crude oil is a complex organic mixture of hydrocarbons and the highest concentration of dissolved hydrocarbons is in the water accommodated fraction which usually consists of aliphatic compounds and aromatic hydrocarbons. Crude oils from different geological formations vary in composition, yet most crude oils contain a polycyclic aromatic hydrocarbon (PAH) fraction that would be expected to produce toxic effects on developing fish embryos and larvae. Exposures to relatively higher PAH concentrations could cause embryonic heart failure and death soon after fish hatch into free-swimming larvae [11, 12]. PAHs are of greatest concern for the marine biota, with genotoxic, carcinogenic or reproductive effects, and could be transferred in the marine food web. Crude oil pollution can be deadly to fish, shellfish and other marine life, particularly if large numbers of fish eggs or larvae are exposed to the oil. The results of numerous studies have shown that many fish species and other organisms may be influenced by organic pollutants, like crude oil residues, that may interfere with endocrine and physiological mechanisms. Understanding how a pollutant affects organism embryonic development is key to determining the risk of the pollutant to the marine environment. Increasingly serious marine oil pollution makes it important to choose a suitable marine fish model for marine ecotoxicological studies. Cardiovascular system is one of the first embryos functional organs and physiological function of the embryonic heart is more likely to be affected by external environmental oil pollutants. The collective effects of oil during embryonic and larval stages can influence the structure and function of the adult fish heart in ways that permanently reduce cardiac performance, potentially leading to delayed mortality [1, 8, 14].

Marine medaka *Oryzias melastigma* originates from the coastal waters and fresh waters of Pakistan, India and Thailand. In classification, *O. melastigma* belongs to the order Beloniformes, family Adrianichthyidae and genera Oryzias. Marine medaka possesses strong environmental tolerance. This organism is capable of adapting to a wide range of temperatures and salinity. It has a short generation time (2-3 months). The relatively large eggs and transparent color simplify experimental observations and operations, such as observing developmental changes during each stage of growth. All of these advantages enhance the potential of *O. melastigma* to be a competent model organism in marine ecotoxicology. These characteristics make it available to culture on a large scale under laboratory conditions as well. The marine medaka and their embryos have become important potential marine fish model organisms for marine ecotoxicology studies due to the advantages of simple culture conditions, typical physiological features and abundant genetic information (http://europepmc.org/articles/PMC3958766 - B11) [2, 4, 10]. The developing fish heart is known as a sensitive target organ for the toxic effects of crude oil. It has been proved that cardiac function was a good model to evaluate the toxicity of environmental pollutants, but few paper report the comparative toxicity of different crude oils for cardiac structure and function of marine medaka *O. melastigma* embryo.

To investigate the impact of different crude oils exposure upon marine fish embryo development and to determine whether different crude oils compositions produce common or distinct toxic effects, the marine medaka embryos are employed as experimental materials for the rapid detection and evaluation of the toxic effects of heavy crude oil and bonny light crude oil in the present study. As a response target cardiac function alterations of medaka embryo to crude oil exposure were focused on. The effects of different crude oil WAF on the developing hearts of medaka embryos were studied and analyzed using microscope and stereomicroscope, including heart rate, heart morphology and blood circulation.

Materials and methods

Preparation of crude oil water accommodated fractions

The heavy crude oil and bonny light crude oil used in the present study were provided by the Organization of North China Sea monitoring center. Crude oils and filtered seawater were mixed at a volume ratio of 1:20 and magnetic stirrer were used to facilitate the dissolution of the crude oils for 24 hours and left to settle for 6 hours at room temperature and dark condition. The aqueous phase of crude oils used as the test stock solution was collected from the bottom without disturbance of the oil slick remaining on the surface by a siphoning device and concentration of the WAF was determined by ultraviolet spectrophotometry according to the specification for marine monitoring GB/T17378-2007 in National Marine Environmental Monitoring Center.

Test organisms

The experimental organism marine medaka *O. melastigma* provided by Dr. Mu Jingli of the National Marine Environmental Monitoring Center were reared in flow ecological farming systems with 30 salinity seawater, 25°C and 14 hours light and 10 hours darkness cycle. To ensure the synchronization of development of medaka embryo, all embryos materials used in the present study were collected in the 3-5 hours post spawning. The stereomicroscope and microscope were used to check fertilization and development of embryos. Morphology and heart rate of embryonic heart at different development stage were observed and recorded using microscope to determine optimum embryos stage as a model of cardiac toxicity.

Exposure conditions

The static acute exposures experiment were carried out in 25°C air-conditioned room and pH, dissolved oxygen and salinity values of the WAF dilution were adjusted to 8.0, 6.0 and 30 respectively. Four exposure concentrations of crude oils WAF and a control treatment were tested with three replicates for each exposure and control treatments. The crude oils WAF was serially diluted with clean filtered natural seawater to the desired concentration. Medaka embryos were assigned to exposure and control treatments for 24 h in 20 mL scintillation flask (Corning Incorporated, USA). For each exposure concentration 30 medaka embryos were randomly distributed into 3 scintillation flask with 10 individuals in each one.

Statistical analyses

Statistical analyses of results were performed using software IBM SPSS statistics 20. The one-way ANOVA was used to determine whether there were significant differences between independent groups.

Results

Selection of embryo development stage for cardiac toxicity model

We characterized the timing and general morphological sequence of embryo development. Medaka embryo was transparent during the early development stage and heartbeat of embryo could be observed using microscope and stereomicroscope since 4 days post-fertilization (dpf). Heartbeat was pretty weak and heart rate was relatively low at that development stage. From 5 dpf to 12 dpf embryos heartbeat become more and more powerful and the heart rate begin to accelerate till the embryos hatched (Fig. 1 and Fig. 2). The heart rate tends to be stable and was relatively less pigmentation in embryonic medaka during 5 dpf to 7 dpf stage. The 6 dpf medaka embryo was chosen as the cardiac toxicity model in the present study.

Effect of heavy crude oil WAF on embryonic cardiac function

Results showed that cardiac function of marine medaka embryos was clearly affected by exposure to heavy crude oil WAF. Significant differences in cardiac structure and function of medaka embryo were found between high exposure and control groups after 24 hours exposure. At highest exposure concentrations (100% heavy crude oil WAF, i.e. 7.2 mg/L), swelled and enlarged heart morphology after 24 h exposure to WAF was found. The mixture of chemicals in the crude oil slows the embryos heart rates, reduces cardiac contractility capability and causes irregular heartbeats. Compared with control group, lower and poor blood circulation and accumulation of blood around the medaka embryo heart area occurred in most exposure groups. On the contrary, there no significant differences was found between controls and low exposure group (0.9 mg/LWAF) in cardiac function after 24 h exposure to heavy crude oil WAF.

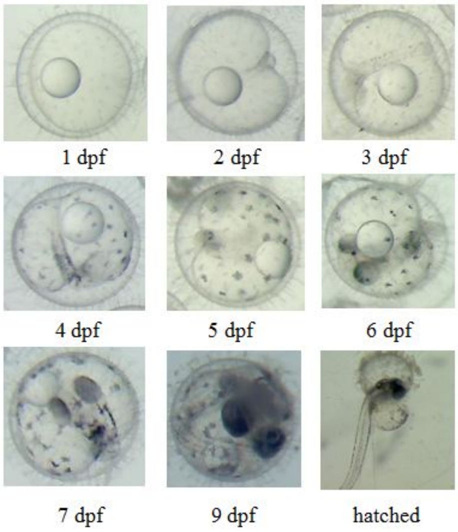

Fig. 1 Embryo development stage of medaka

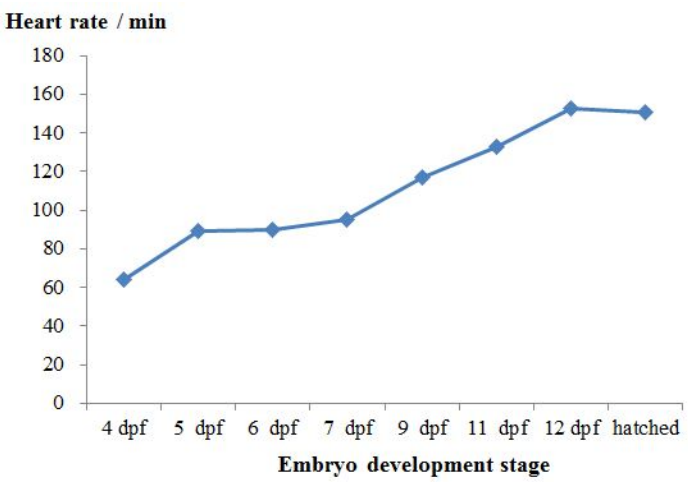

Fig. 2 Heart rate of medaka at different development stage

The most affected physiological parameter in cardiac function of medaka embryo is heartbeat and heart rate. There some significant declines of heart rate were observed at relatively high exposure concentration of heavy crude oil WAF. Compared with control group, the embryos heart rate dropped by 20.70%, 21.32% and 24.03% at 1.8 mg/L, 3.6 mg/L and 7.2 mg/L exposure treatments, respectively.

Table 1. Cardiac toxic effect of different concentration heavy crude oil WAF

WAF concentration	Heart morphology	Heart beat	Blood circulation
7.2 mg/L	Swelled and enlarged	Slowed and weak	Lowered
3.6 mg/L	No significant change	Slowed	Lowered
1.8 mg/L	No significant change	Slowed	Lowered
0.9 mg/L	No significant change	No significant change	No significant change
Control	Normal	Normal	Normal

Effect of bonny light crude oil WAF on embryonic cardiac function

Results showed that cardiac function of marine medaka embryos was only affected by highest concentration (100% bonny light crude oil WAF, i.e. 9.8 mg/L) of bonny light crude oil 24 hours exposure. Statistics analysis showed that there was significant difference in heart rate between highest concentration treatment and control group. Furthermore, compared with control group, there no swelled and enlarged heart morphology of medaka embryo was found in exposure groups (Fig. 3 and Fig. 4).

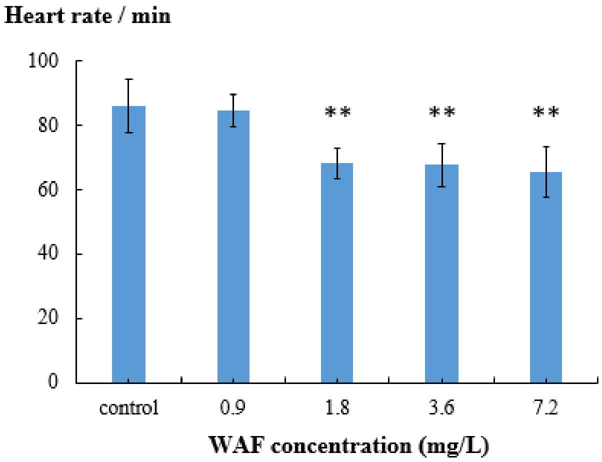

Fig. 3 Mean heart rates of medaka embryo in control and heavy crude oil groups

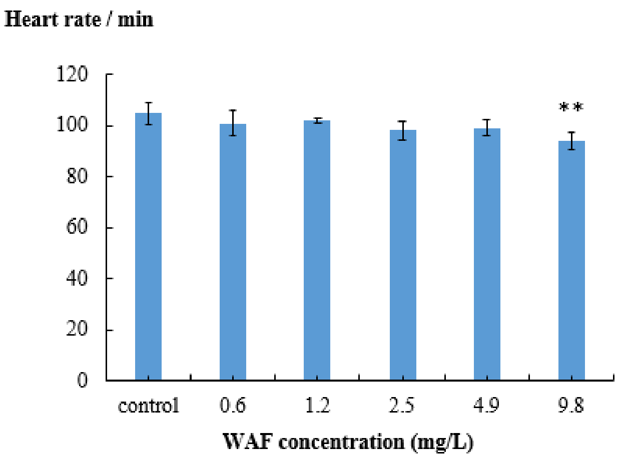

Fig. 4 Mean heart rates of medaka embryo in control and bonny light crude oil groups

Discussions

It is well known that exposure to oil-related chemicals may lead to serious physiological and biochemical changes or damage to several organs or tissues in the marine organisms. It has been proved that fish cardiac function of early development stage is a suitable model to study the toxicity of oil-related chemicals and is pretty sensitive to the exposure of crude oil [6, 9, 13, 15]. Crude oil contains mixtures of polycyclic aromatic hydrocarbons, which adversely affect heart development by slowing the heartbeat or causing an uncoordinated rhythm. Some studies have showed that toxicity effects on a developing fish heart are severe which could permanently impair cardiac function and life performance at later life stages. When exposed to crude oil transiently fish can suffer subtle and chronic changes in heart rhythm in that the heart muscle becomes deformed. Severely affected fish with deformed heart muscle are likely to die soon after hatching.

The embryo of this species has been identified as an important tool for toxicology investigations. Many studies have been conducted on marine medaka, especially in terms of their physiological, biochemical, and molecular responses after exposure to contaminants. It is very important to choose which development stage of embryos as an evaluation model when we focus on cardiac toxicity effect of crude oil on medaka embryos. The sensitivity and stability of target organ or tissue are usually different during different development stages. In the present study the heart rate of marine medaka embryo is relatively stable and pigment is less in the embryo surface during 5 dpf to 7 dpf stage. So it is very convenient to observe and record the subtle changes of embryonic cardiac developmental morphology and heart rate during these stages.

In the present study, the cardiac function of marine medaka embryos was clearly affected by exposure to heavy crude oil. The affected physiological parameters included heart morphology, heart rate and blood circulation. Results showed that the higher the concentration posed more serious cardiotoxic effects. These findings indicate that cardiac function of marine medaka embryo may be used as a toxicity model and biomarker for oil exposure induced toxic effect. In addition to a slowing heartbeat, crude oil exposure resulted in an irregular arrhythmia. The results showed that both crude oils caused similar cardiotoxicity in marine medaka early stage embryos and the cardiotoxic effect of heavy crude oil was more serious than that of the bonny light crude oil. The remarkably consistent morphological and physiological responses to different crude oil indicate that the core mechanisms of PAH-induced cardiotoxicity are conserved. This research has strong implications for marine ecological damage assessment after oil spills.

Conclusion

The results above showed that there were obvious differences in toxicity between heavy crude oil and bonny light crude oil. The cardiotoxic effect of heavy crude oil was more serious than that of the bonny light crude oil. Significant differences in cardiac structure and function of medaka embryo were found between exposure and control groups. The heavy crude oil WAF slowed down the embryos heart rates, reduced cardiac contractility capability and causes irregular heartbeats. Both crude oils caused similar cardiotoxicity in marine medaka early stage embryos and the impacts of heavy crude oil on marine fish embryos cardiac injury phenotype are consistent with the bonny light crude oil. The physiological mechanisms by which crude oil affects the cardiac structure and function need to be studied further.

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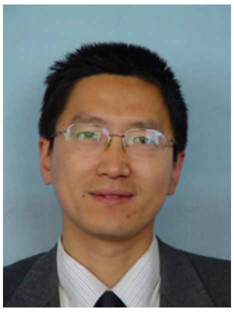

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