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Beneficial effect and potential molecular mechanism of chloroquine on sperm motility and fertilizing ability in yellow catfish

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ABSTRACT

Sperm motility is a critical determinant of male fertility in vertebrates including fish species. Chloroquine, an autophagy inhibitor, has been reported to own debated influence on sperm quality of mammalians. The objectives of this study were to determine the effect of chloroquine on sperm motility and fertilizing efficiency in yellow catfish, an important freshwater fish species in China. Gradient doses of chloroquine were intraperitoneally injected into yellow catfish that resulted in an increasing expression level of LC3-II and inhibition of autophagy. Subsequently, various sperm parameters were assessed with the computer-assisted sperm analysis (CASA) system. Chloroquine injection significantly increased straight line velocity (VSL), curvilinear velocity (VCL) and average path velocity (VAP) of sperm compared with the control. Consequently, 1 μM chloroquine/g body weight was revealed to be an optimal dose for in vivo treatment, which led to elevation of fertility rate, whereas no effect on the hatching rate. The comparative transcriptome analysis between chloroquine-treated and control testes were conducted in order to figure out the possible molecular mechanism of chloroquine to regulate sperm motility. Interestingly, Gene Ontology (GO) analysis indicated that chloroquine treatment not only inhibited autophagy pathway but also significantly reduced toll-like receptor signaling pathway, suggesting a possible trade-off between male reproduction and immunity. KEGG analysis showed up-regulation of many pathways including PI3K-AKT signaling pathway which has been reported to be correlated with spermatogenesis and sperm maturation in yellow catfish. Our data reveals a potential trade-off between reproductive traits and immune function, and suggests that the application of chloroquine could improve sperm quality and fertilization efficiency in broodstock fish.

Statement of relevance: Our research demonstrated that an optimal dose of chloroquine could efficiently inhibit autophagy and improve sperm motility and fertilization efficiency. The comparative transcriptome analysis between chloroquine-treated and control testes were conducted in order to figure out the possible molecular mechanism of chloroquine to regulate sperm mobility.

1. Introduction

Over the past decades, the fish farming industry paid more attention to the quality of eggs and larvae rather than to that of sperm, although the quality of both gametes is associated with fertilization success, growth and survival of the progeny (Rurangwa et al., 2004). Under the pressure from sexual selection and sperm competition, interest for sperm quality analysis has been aroused to increase the reproductive success and maintain the genetic diversity of the lineages (Mehlis et al., 2015; Galeotti et al., 2012). Multiple quality indexes including motility, spermatocrit, viability and fertilization success have been used to define the quality of the sperm. Sperm motility is a quantitative trait and a critical determinant of fertility (Rurangwa et al., 2004), and it can be evaluated by Computer-assisted sperm analysis (CASA) in many vertebrates including fish species (Gallego et al., 2013).

A number of small peptides and compounds have been reported to regulate sperm motility, mainly by increasing [Ca^{2+}] and cAMP level in mammalians. Also, many small molecules have been shown to be inducers of the acrosome reaction, including glycine, prostaglandin E, cholesterol sulfate, acetylcholine, g-aminobutyric acid (GABA), ATP and other glycans (Yoshida et al., 2008). In fish species, sperm motility is induced when spermatozoa are released from the male genital tract into the aqueous environment (Alavi and Cosson, 2006). External environmental factors, such as osmotic pressure, ionic and gaseous components influence fish spermatozoa motility and activation (Dzyuba and Cosson, 2014). Recent studies showed that dietary supplementation with n-3 polyunsaturated fatty acids, astaxanthin could improve sperm motility

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and fertilization rate (Koprucu et al., 2015; Tizkar et al., 2015). However, few reports existed that explain the molecular mechanism corresponding to the improvement of sperm motility.

Chloroquine is an anti-malaria drug and well known as an autophagy inhibitor (Fang et al., 2013). The effects of chloroquine on sperm are time and dose-dependent, as short-term or low-dose treatment resulted in an increase in sperm motility and fertility in mammalians (Norman and Gombe, 1975; Egbunike, 1982), whereas long-term or high-dose treatment resulted in a dose dependent decrease in sperm motility and fertility (Adeeko and Dada, 1998; Okanlawon et al., 1993). However, molecular mechanisms of chloroquine for increasing sperm motility and successful fertilization remain unclear. All-male yellow catfish has been successfully produced since males grow faster than females (Mei and Gui, 2015). To investigate whether autophagy affects sperm motility and fertilization efficiency in male yellow catfish, chloroquine was administered intraperitoneally to adult fish, and the sperm motility was evaluated by CASA. In addition, we utilized comparative transcriptome analysis to identify differently expressed genes and pathways between chloroquine-treated and control testis. Hopefully, our findings would reveal the potential effect and molecular mechanism of chloroquine on sperm motility and fertilizing ability in fish species.

2. Materials and methods

2.1. Experimental animals and chloroquine treatment

One-year-old sexually mature yellow catfish with similar size were collected from the farming place at Jiangxia, Wuhan, Hubei province, China. All fish were acclimatized in the laboratory facility for one week as previously described (Xiong et al., 2015). The experimental operations were conducted as the requirement of the institution animal care and use committee of Huazhong Agricultural University.

Chloroquine (sigma–Aldrich) was dissolved in 1 × PBS and injected into healthy male individuals (100 ± 2.5 g) behind the pectoral fin, at a dose of 50, 100, 500 and 1000 μM per 100 g, respectively. The control groups were injected only with 1 × PBS solution. For each of the independent experiments, four male yellow catfish per group were used.

2.2. Evaluation of sperm motility and in vitro fertilization efficiency

Sperm samples were collected from control and treated groups 24 h after the administration of 1 μM chloroquine/g body weight. Sperm concentration was assessed by conventional hemocytometry using a phase-contrast microscope (400 × magnification) according to a previous study (Gennotte et al., 2012). In each case, sperm samples were diluted with sperm preservative fluid (63 mM NaCl, 19 mM KCl, 1.3 mM $CaCl_2$, 4.7 mM $MgSO_4 \cdot 7H_2O$, 2.5 mM $NaHCO_3$, pH 7.4) to a concentration of 6×10^8 sperms/mL. Total sperm number of each individual was calculated by multiplying sperm concentration and sample volume. The final concentration of sperm is about 550 cells/μl activation solution. Spermatozoa motility and kinematic parameters were quantified by CASA II using Animal Motility Software Manual Version 1.4 (Hamilton-Thorne Research, Beverly, USA) in which camera speed is 60 frames/s as previously described (Kasimanickam et al., 2007; Kwon et al., 2013). The 10 × phase-contrast objective was chosen to analyze the spermatozoa, and the movement of sperms in each sample (1 mL activation solution) was recorded from at least three randomly selected fields for three times. By convention, all sperm present or entering the field in the first 10 frames are identified and counted during the acquisition.

The ratio of egg/sperm has been optimized by preliminary experiments to investigate fertilization efficiency of sperm when treated with 100 μM chloroquine or not. In particular, each group containing 2.5 g eggs (about 400 eggs per gram) was respectively artificially inseminated with 0, 10, 20, 30, 40 and 50 μL sperm at a concentration of 8×10^6 sperms/mL. Embryos were incubated with aerated water and at room temperature. The percentage of fertilized and unfertilized embryos were recorded and calculated at 16 h post fertilization.

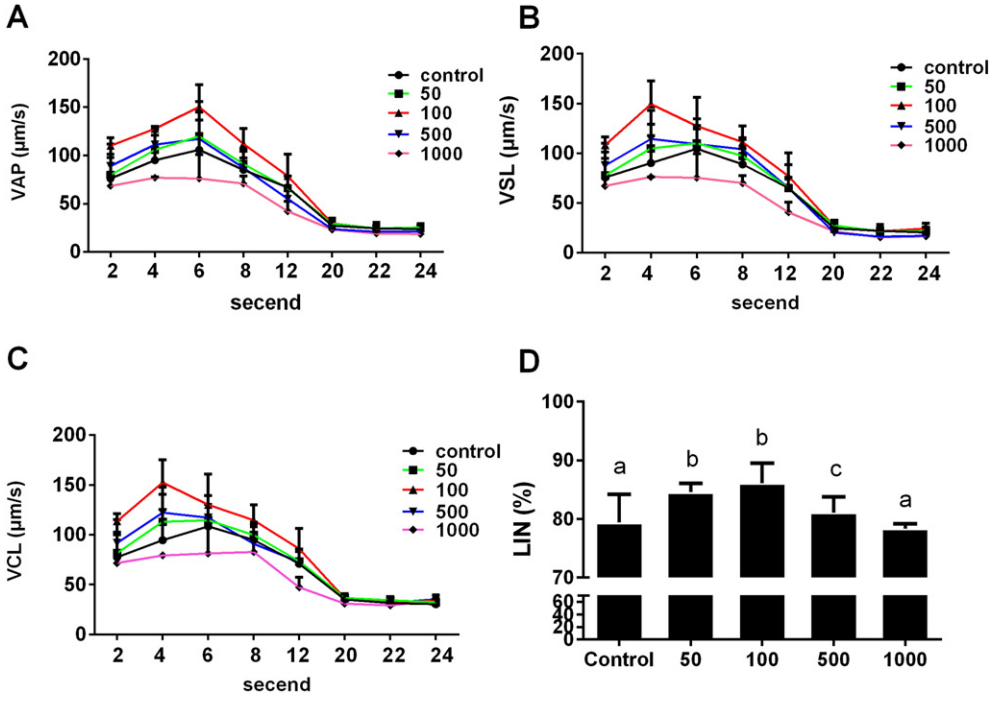

Fig. 1. Comparisons of semen kinematic parameters between chloroquine-treated groups and control group in yellow catfish. Different doses (0, 50, 100, 500 and 1000 μM/100 g body weight) of chloroquine were used. Four kinematics parameters including average path velocity (A), straight line velocity (B), curvilinear velocity (C), and linearity (LIN) were assessed with the computer-assisted sperm analysis (CASA) system.