Alcohol xxx (2016) 1–7

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Reversible loss of reproductive fitness in zebrafish on chronic alcohol exposure

Pooran Singh Dewari [1], Funmilola Ajani [2], Gopal Kushawah, Damera Santhosh Kumar, Rakesh K. Mishra*

Centre for Cellular and Molecular Biology, Council of Scientific and Industrial Research, Uppal Road, Hyderabad 500007, India

ARTICLE INFO

Article history:
Received 5 May 2015
Accepted 10 November 2015

Keywords:
Alcohol
Zebrafish
Chronic alcohol exposure
Fecundity
Anxiety

ABSTRACT

Alcoholism is one of the most prevalent diseases in society and causes significant health and social problems. Alcohol consumption by pregnant women is reported to cause adverse effects on the physical and psychological growth of the fetus. However, the direct effect of chronic alcohol consumption on reproductive fitness has not been tested. In recent years, the zebrafish (*Danio rerio*) has emerged as a versatile model system to study the effects of alcohol on behavior and embryonic development. We utilized the zebrafish model system to address the effect of chronic alcohol exposure (0.5% alcohol in the holding tank for 9 weeks) on reproductive capacity. We found a dramatic decrease in fecundity, measured by counting the number of eggs laid, when at least one of the parents is subject to chronic alcohol exposure. Interestingly, a 9-week alcohol withdrawal program completely restored the reproductive capacity of the treated subjects. In agreement with observations on fecundity, the chronic alcohol exposure leads to increased anxiety, as measured by the novel-tank diving assay. Conversely, the withdrawal program diminished heightened anxiety in alcohol-exposed subjects. Our results highlight the adverse effects of chronic alcohol exposure on the reproductive capacity of both males and females, and underscore the utility of the zebrafish model system to understand the biology of chronic alcoholism.

Introduction

Alcohol dependence or alcoholism is a disease that is defined as repeated, uncontrolled consumption of alcohol (ethyl alcohol, ethanol, EtOH) despite its detrimental effect on the health and socioeconomic status of both drinkers and their family members. Globally, in 2012, about 3.3 million deaths were caused by alcohol consumption, which corresponds to 5.9% of all deaths (World Health Organization, 2014). Alcohol consumption causes more than 200 health conditions, most notably liver cirrhosis, cancer, depression, anxiety, cardiovascular diseases, and diabetes. In addition to health outcomes, alcohol consumption has serious socioeconomic consequences on the drinker's family members and society at large. Alcohol consumption by a woman during pregnancy has serious consequences for the health of the fetus, including growth defects, craniofacial abnormalities, and central nervous system damage, collectively called fetal alcohol spectrum disorders (FASD) (Muralidharan, Sarmah, Zhou, & Marrs, 2013).

Zebrafish, *Danio rerio*, have emerged as a powerful model system to understand vertebrate development, behavior, and human diseases (Norton & Bally-Cuif, 2010; Santoriello & Zon, 2012; Veldman & Lin, 2008). The prolific production of eggs, external fertilization, nearly transparent nature of developing embryos, genetic similarity to humans, and ease of genetic manipulation make them an ideal system to study vertebrate biology. Drug administration is fairly simple, as hydrophilic drugs can be added directly to the water and their effects on embryonic or adult fish can be studied. Having these advantages over other model organisms, zebrafish are being successfully used as a model system to understand the biology of various human diseases including different cancer types, metabolic diseases, muscular dystrophy, and disorders related to central nervous system, heart, and kidney (Kari, Rodeck, & Dicker; 2007; Santoriello & Zon, 2012; Seth, Stemple, & Barroso, 2013). In recent times, zebrafish have drawn attention as a tractable vertebrate model system to study alcoholism (Gerlai, Lahav, Guo, & Rosenthal, 2000; Gerlai, Lee, & Blaser, 2006; Marrs et al., 2010). Simple behavioral paradigms are now available that can be used to measure the effect of alcohol on zebrafish (Gerlai

* Corresponding author. E405, Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, India. Tel.: +91 40 2719 2658; fax: +91 40 2716 0591.
E-mail address: mishra@ccmb.res.in (R.K. Mishra).
[1] Present address: MRC Centre for Regenerative Medicine, SCRM Building, 5 Little France Drive, Edinburgh, UK.
[2] Present address: University of Ibadan, Ibadan, Nigeria.

http://dx.doi.org/10.1016/j.alcohol.2015.11.006
0741-8329/

et al., 2000; Herculano & Maximino, 2014; Maximino, de Brito, da Silva Batista et al., 2010). Various studies have documented the dreadful effects of both chronic and acute alcohol exposure on embryonic development and adult behavior. Development of zebrafish embryos in an alcohol-containing medium mirrors FASD and results in craniofacial abnormalities, small eyes, enlarged body cavity, and behavioral impairment (Ali, Champagne, Alia, & Richardson, 2011; Bilotta, Barnett, Hancock, & Saszik, 2004; Muralidharan et al., 2013). At lower doses, both acute and chronic alcohol exposure have an anxiolytic effect on adult zebrafish (Dlugos & Rabin, 2003; Egan et al., 2009), the response being strain-dependent, suggesting that the genotype influences response to ethanol (Dlugos & Rabin, 2003; Gerlai, Ahmad, & Prajapati, 2008). Interestingly, the response to anxiolytes (e.g., ethanol) also depends on housing conditions (Parker, Millington, Combe, & Brennan, 2012). Chronic alcohol exposure is associated with increased stress level, organ failure, tissue damage (Adachi & Ishii, 2002; Caimi, Carollo, & Lo Presti, 2004; Harper & Matsumoto, 2005; Massey et al., 2015), oxidative stress (Bondy, 1992; Fernández-Checa, Kaplowitz, Colell, & García-Ruiz, 1997), high cortisol level and decreased hippocampal volume (Adinoff, Ruether, Krebaum, Iranmanesh, & Williams, 2003; Beresford et al., 2006), neurodegenerative diseases (Beresford et al., 2006; Bowden, Crews, Bates, Fals-Stewart, & Ambrose, 2001; Crews & Braun, 2003), disturbed onset of puberty, perturbed endocrine system (Emanuele & Emanuele, 1997; Richardson, Lee, O'Dell, Koob, & Rivier, 2008), suppressed immune system (Happel & Nelson, 2005), and severe defects in the circadian clock (Brager, Ruby, Prosser, & Glass, 2010; Sarkar, 2012). Continuous long-term chronic alcohol exposure also shows a shift in brain decision-taking function in the region associated with habit development (DePoy et al., 2013).

Although various studies have looked into the effect of acute and chronic alcohol exposure on zebrafish behavior, none so far have addressed the effect on reproductive capacity (fecundity). Therefore, we undertook this study to assess the effect of chronic alcohol exposure on zebrafish fecundity. Our results outline the negative effect of chronic alcohol exposure on fecundity. Complete recovery after a withdrawal regime points out that the negative outcomes of chronic alcoholism can be reversed, suggesting a better prognosis. We propose that the zebrafish can be used as a model system to study reproductive dysfunction caused by chronic alcohol abuse and also for screening of drugs to alleviate the negative effects, *viz*., increased anxiety and reduced fecundity.

Materials and methods

Experimental design

The schematic representation of the experimental set-up is given in Fig. 1. One-hundred twenty naïve wild-type (short fin, '*AB*' strain) *D. rerio* (60 males, 60 females) were separated into four groups, representing male control, male alcohol-exposed, female control, and female alcohol-exposed groups. Thus, each group was composed of 30 fish, exclusively either male or female, maintained in a 20-L water tank. For the chronic alcohol exposure program, fish were transferred every afternoon into a new tank containing 0.5% ethanol and maintained in the same tank for the next 24 h. Experimental conditions of automated light/dark cycle (14/10 h), temperature of 28 °C, water salinity of 3 g of sea salt per liter, and aeration by air bubbling were maintained. This way, fish were exposed to 0.5% ethanol for 9 weeks. Fish in the control group were also transferred each afternoon to a new tank but without ethanol in the medium. From the 5th week of alcohol treatment to the 9th week, fish were bred to measure fecundity. Fish in the four groups were bred in four different combinations – both partners control, only male alcohol-exposed, only female alcohol-exposed, and both sexes alcohol-exposed. Because alcohol-exposed fish were very vulnerable to handling stress and did not lay eggs if handled just

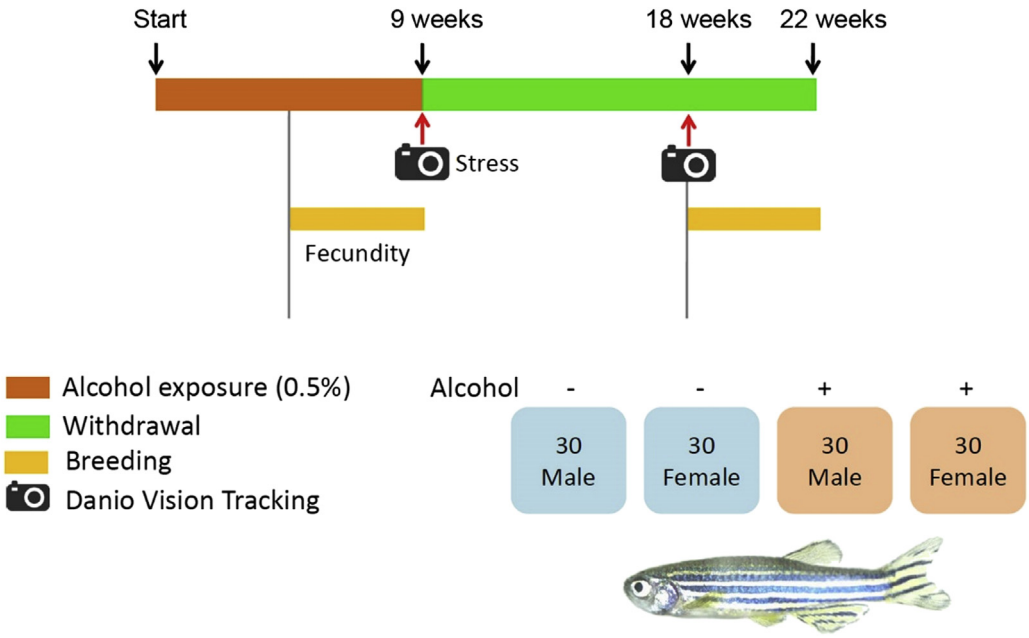

Fig. 1. Schematic representation of the experimental plan. For chronic alcohol exposure, zebrafish were maintained for 9 weeks in holding tanks containing 0.5% v/v ethanol. To measure fecundity, fish were bred in different combinations from the 5th to 9th week of alcohol exposure. After the termination of alcohol treatment, fish were maintained in a holding tank without ethanol (withdrawal period) for the next 9 weeks. After the withdrawal program, fish were bred for the next 4 weeks to measure fecundity. The novel-tank diving assay, the behavioral test denoted by the camera icon, was performed to measure anxiety at the end of both the chronic alcohol treatment (9th week) and withdrawal program (18th week).