

# Metabolic changes in the acute alcohol induced zebrafish brain

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## INTRODUCTION

The zebrafish has been a popular subject of embryology, genetic research as well as neurobiology for the past three decades. Nevertheless, very few zebrafish metabolism studies using nuclear magnetic resonance spectroscopy existed for mutation or drug screening purposes. Alcoholism is one of the biggest and costliest diseases whose mechanisms are not well understood. [1,2] Model organisms such as the zebrafish may be utilized in this line of research. Although several alcoholic zebrafish researches such as behavior test or chromatography have performed [1], the NMR study has not. Therefore, the purpose of this study was to investigate the metabolic alteration in acute alcohol induced zebrafish brain using NMRS in this study.

## MATERIALS AND METHODS

Adult zebrafish (*Danio rerio*) of the AB strain were used. In order to investigate the acute alcohol effect by the variation of alcohol concentration, zebrafish were treated in the four alcohol dissolved tanks in the presence of the 0.00% (control; n=10), 0.25% (n=10), 0.50% (n=10) and 1.00% (n=10) (v/v). For 1h, fish were exposed acute alcohol in the four tanks. As soon as fish were removed from the alcohol dosing tanks, their whole brains removed from the skull and frozen in liquid nitrogen. The metabolite powders were obtained from fish brains using methanol-chloroform (M/C) water extraction methods of Le Belle et al. [3]. NMR samples were made as dissolving metabolite powders in 99.9% D<sub>2</sub>O containing 0.75% TSP solvent. Spectroscopy was performed at 25°C with a Varian 500 MHz FT-NMR spectrometer. One-dimensional NMRS parameters were as follows: CPMG spin echo pulse, relaxation/saturation delay/pre-saturation time = 2.0/2.0 s (effective TR = 4 s), inter-pulse delay = 1 ms (effective TE = 2 ms), complex number = 8192, spectral width = 8000 Hz, and number of scans = 256. The acquired spectra were analyzed using Mestrenova-TM, which post-processed experimental spectra. Metabolite concentrations were calculated using internal referencing quantification (TSP peak reference).

## RESULTS

The metabolite concentrations of zebrafish brain were obtained as showing Fig.1. Statistical analysis confirmed this observation. ANOVA (Tukey HSD analysis) showed a significant time effect for all neurochemicals tested: glutamate (Glu) F(3,39)=4.624, p<0.01; scyllo-Inositol (sIns) F(3, 39) = 2.881, p < 0.05; myo-Inositol (mIns) F(3,39) = 7.380, p < 0.005; [Glu]/[tCr] F(3,39)=7.122, p < 0.005; [sIns]/[tCr] F(3, 39) = 3.162, p<0.05; [mIns]/[tCr] F(3, 43) = 6.887, p<0.005. Fig.2 shows that the concentrations relative to tCr of sIns, mIns and Glu also were significantly different among four groups. The concentration of Glu ([Glu]) and [Glu]/[tCr] was reduced by acute alcohol exposure. [sIns] and [sIns]/[tCr] increased while [mIns] and [mIns]/[tCr] decreased.

## DISCUSSION

In this work, the metabolite concentrations of zebrafish brain as our result were good agreement with previous article. [4] As well as this study demonstrated that sIns is an excellent biomarker of brain metabolism in acute/chronic alcohol effect of zebrafish as the published report. In this study, NAA, Tau and Cho were not significantly altered because of the use of whole brain. However, we demonstrated that acute alcohol exposure decreases Glu and mIns, and increases sIns in zebrafish brain. These results support that the induced alcohol in a brain reduces Glu level for detoxification of blood and works a disturbance the pathway from sIns to mIns.[2]

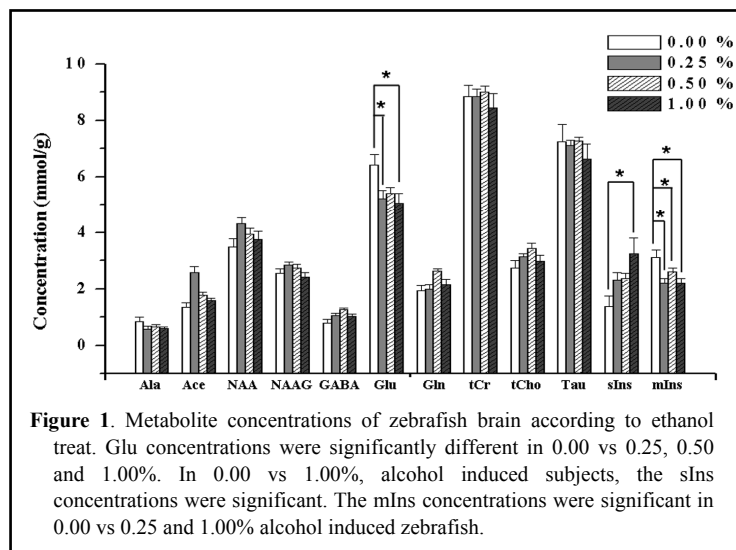
We revealed in this study that the combination of zebrafish and NMRS was suitable to research the alcohol effect for brain system such as alcohol withdrawal effect, alcohol addiction.

## ACKNOWLEDGEMENTS

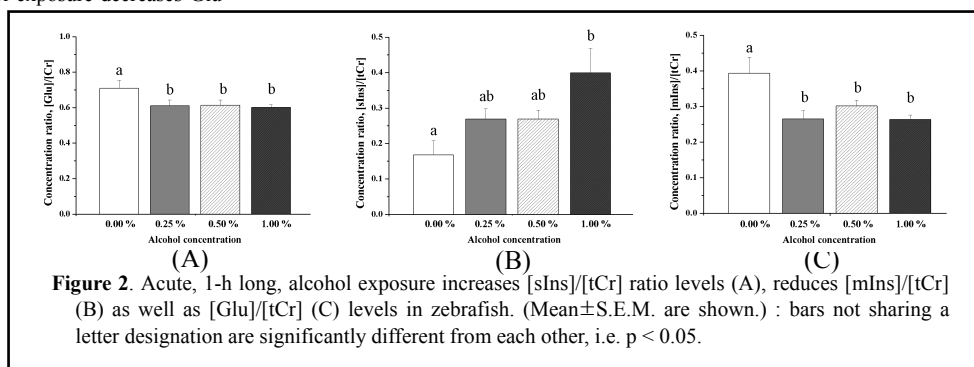
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**Figure 1.** Metabolite concentrations of zebrafish brain according to ethanol treat. Glu concentrations were significantly different in 0.00 vs 0.25, 0.50 and 1.00%. In 0.00 vs 1.00%, alcohol induced subjects, the sIns concentrations were significant. The mIns concentrations were significant in 0.00 vs 0.25 and 1.00% alcohol induced zebrafish.



**Figure 2.** Acute, 1-h long, alcohol exposure increases [sIns]/[tCr] ratio levels (A), reduces [mIns]/[tCr] (B) as well as [Glu]/[tCr] (C) levels in zebrafish. (Mean±S.E.M. are shown.) : bars not sharing a letter designation are significantly different from each other, i.e. p < 0.05.