Protection role of resveratrol against alcohol-induced heart defect in zebrafish embryos

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To the Editor: Alcohol-induced heart defect is one of the most important clinical manifestations of fetal alcohol spectrum disorder (FASD), characterized by atrioventricular septal defect and arterial conical deformity.[1] Resveratrol, a polyphenol component of the traditional Chinese herb *Polygonum cuspidatum*, which is mainly extracted from its rhizome, is known to exhibit beneficial effects on the cardiovascular system. For example, resveratrol has beneficial effects on heart dysfunction, myocardial hypertrophy, and pressure overload. Moreover, resveratrol was found to inhibit platelet aggregation, prevent atherosclerosis, and scavenge free radicals.[2] However, the effect of resveratrol on alcohol-induced heart defect during heart formation is still unknown. The unique characteristics of zebrafish embryos, such as their transparent body, *in vitro* fertilization, and short reproductive cycle, make them an attractive tool for cardiovascular research. Moreover, the immersion-based alcohol delivery method used with zebrafish embryos is non-invasive unlike most of the alcohol administration protocols applied in rodent models.[3]

Our study aimed to investigate the protective effect of resveratrol against alcohol-induced heart defect during zebrafish heart formation. This was fulfilled by using alcohol-treated zebrafish fertilized eggs as the model and resveratrol as the intervention, followed by the assessment of heart morphology and function.

Zebrafish (*Danio rerio*; AB strains) was raised in automatic fish housing systems at 28.5°C with 14/10 h light/dark schedule. Zebrafish embryos were obtained from spawning of adult zebrafish. Alcohol solutions (1% and 2%) were prepared from a stock solution of 95% ethanol (Thomas Scientific, Shanghai, China; catalog No. C315H70) diluted with system water. Resveratrol (0.3 g; Vetec company, Beijing, China; catalog No. V900386-5G) was dissolved in 10 L system water to obtain a saturated solution (0.03 g/L).

Within 2 h of spawning, fertilized eggs were collected and divided into six groups (20 embryos/group), and were exposed to the following different combinations of drugs until 3 days post-fertilization (dpf): 0 alcohol + 0 resveratrol (control group), 1% alcohol + 0 resveratrol, 2% alcohol + 0 resveratrol, 0 alcohol + resveratrol (0.03 g/L), 1% alcohol + resveratrol (0.03 g/L), and 2% alcohol + resveratrol (0.03 g/L). At 3 dpf, 10 embryos from each group were randomly selected for morphologic observation and heart rate measurement. Pericardial edema was defined as the exhibition of an abnormal accumulation of fluid around the heart chambers. Body length of larvae was measured by the calibrated reticule of the microscope. Heart rate was measured manually for 1 min in each embryo. Images were digitally acquired with an inverted microscope (Leica Microsystems, Singapore; catalog No. Leica M205C). After measurements, embryos were collected for mRNA extraction and reverse transcription (RT) to cDNA for RT quantitative polymerase chain reaction (RT-qPCR). Three independent experiments were performed in triplicate.

At 3 dpf, total RNA was extracted from the 10 whole embryos using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA; catalog No. 15596-026) according to the manufacturer's instructions. A total of 500 ng of RNA was reverse transcribed to cDNA using PrimeScript RT reagent kit (Takara, Shiga, Japan; catalog No. RR037A). Each real-time PCR amplification reaction was performed with 1 μL of cDNA using Power SYBR Green PCR mix (Applied Biosystems, Foster City, CA, USA) and 0.5 mmol/L of each primer. Primers were synthesized by Sunny

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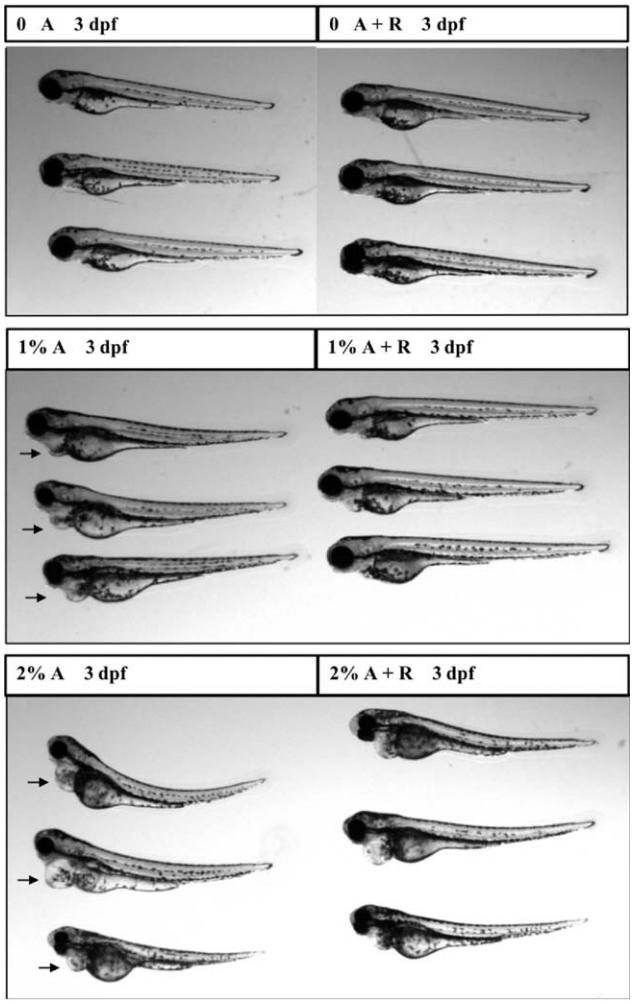

Figure 1: Pericardial edema in zebrafish embryos at 3 days post-fertilization (dpf). Left part shows pericardial edema in zebrafish embryos treated with different alcohol concentrations, and right part shows resveratrol effects (0.03 g/L) on alcohol-induced pericardial edema. Black arrows show the actual location of pericardial edema. A: Alcohol; R: Resveratrol.

Biotechnology Co, Ltd. (Shanghai, China). Following are the detailed primer sequences: *bmpr2b* (Forward: 5′-GGCTCTGCTCACTGCTTCTG-3′, Reverse: 5′-TGCGA-TGGCGTTGTGGTAAC-3′), *hand2* (Forward: 5′- GAG-TTTAGTTGGAGGGTTT CCCCACC-3′, Reverse: 5′-GTAGTGCGAATGGTCGAGCCCG-3′), *tbx5a* (For-ward: 5′- CAGACAAACAGAATGCAGCCGTCA-3′, Reverse: 5′-ACTTTGAAGCTGGGAAACATC CGC-3′), and *β-actin* (Forward: 5′-CGAGCTGTCTTCCCATCCA-3′, Reverse: 5′- TCACCAACGTAGCTAGCTGTCTTT-CTG-3′). Thermal cycling was carried out in LightCycler 480 (Roche, Basel, Switzerland). *β-actin* was used as an internal control for normalization.

All data analyses were performed using the statistical software GraphPad Prism 7.0 (GraphPad, San Diego, CA, USA). Differences in body length and heart rate between alcohol and alcohol with resveratrol groups were assessed by the independent Student *t* test. Gene expression data were analyzed by one-way or two-way analysis of variance. A value of $P < 0.05$ was considered statistically significant.

Obvious pericardial edema, which appeared as a large transparent bubble around the heart, was found in the alcohol-exposed groups, and was more severe with 2% alcohol than that with 1% alcohol. Addition of resveratrol in the 1% alcohol + resveratrol group partly alleviated the pericardial edema compared to the 1% alcohol group. However, morphology was not improved by resveratrol in embryos exposed to 2% alcohol [Figure 1]. Differences in pericardial edema between the different groups did not reach statistical significance. Body lengths were also measured to quantify morphologic changes [Figure 2A]. Compared to untreated control embryos, alcohol-treated embryos had shorter bodies at 3 dpf. Addition of resveratrol significantly recovered body lengths in 1% ($P = 0.046$) and 2% ($P < 0.001$) alcohol-treated larvae.

At 3 dpf, heart rate was found to be higher in alcohol-treated groups compared to the control group. Addition of resveratrol significantly lowered heart rates in 1% alcohol-treated embryos ($P = 0.011$), but had no significant

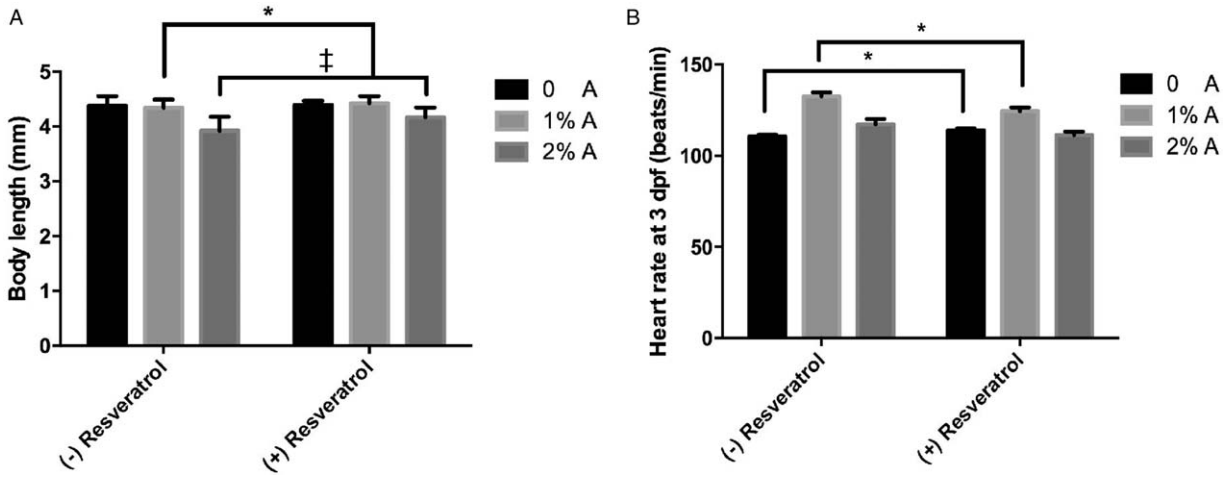

Figure 2: Resveratrol effects on alcohol-induced changes in body length and heart rate. (A) Body lengths of different treated groups at 3 days post-fertilization (dpf). (B) Heart rates of different treated groups at 3 dpf. $^{*}P < 0.05$. $^{‡}P < 0.0001$. A: Alcohol.

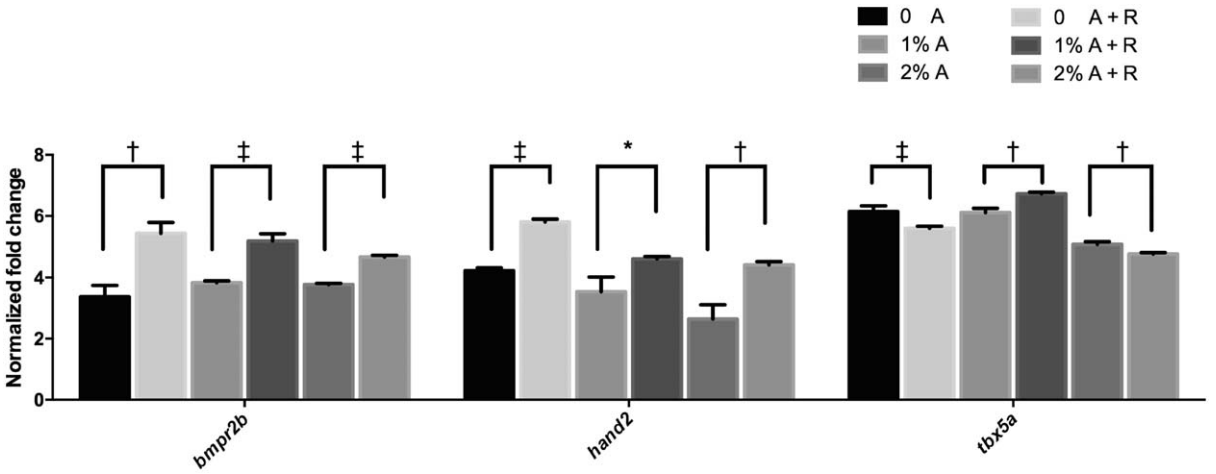

Figure 3: Relative mRNA levels of three heart-related genes, *bmpr2b*, *hand2*, and *tbx5a*, in zebrafish embryos at 3 days post-fertilization (dpf). $^{*}P<0.05$. $^{\dagger}P<0.001$. $^{\ddagger}P<0.0001$. A: Alcohol; R: Resveratrol.

effect on faster heart rates induced by 2% alcohol [Figure 2B].

To further investigate the possible underlying mechanisms for resveratrol effect on the cardiovascular system, the mRNA expression of several heart-related genes (*bmpr2a*, *bmpr2b*, *gata5*, *hand2*, *nkx2.5*, *tbx1*, and *tbx5a*) was determined by RT-qPCR. As shown in Figure 3, resveratrol induced significant changes in the mRNA expression of *bmpr2b*, *hand2*, and *tbx5a* compared to alcohol-treated groups. The other four genes showed no negative results. Regarding *bmpr2a*, addition of resveratrol to alcohol-treated embryos significantly elevated its mRNA expression compared to 1% alcohol ($P<0.001$) and 2% alcohol ($P<0.001$) groups. Similarly, resveratrol treatment significantly increased *hand2* mRNA expression in alcohol-treated embryos ($P=0.019$ and 0.003 compared to 1% and 2% alcohol groups, respectively). Regarding *tbx5a*, its mRNA level was increased in the 1% alcohol + resveratrol group compared to that in the 1% alcohol group, while it was slightly decreased in the 2% alcohol + resveratrol group compared to that in the 2% alcohol group.

This study demonstrated that resveratrol had a protective effect against alcohol-induced morphologic and functional abnormalities in zebrafish embryos such as shortening of body length, pericardial edema, and tachycardia. Exposure to resveratrol was also found to alter the expression of some heart-related genes such as *bmpr2b*, *hand2*, and *tbx5a* at 3 dpf. Our study provided evidence regarding the potential protective effect of *Polygonum cuspidatum* against FASD.

In the present study, we demonstrated that resveratrol could protect against alcohol-induced morphologic changes. Short body lengths and pericardial edema were observed in zebrafish embryos exposed to alcohol, as shown in a previous study.[4] Resveratrol was found to alleviate pericardial edema and tachycardia induced by 1% alcohol, but had no significant effect on the same phenomena induced by 2% alcohol. This possibly means that changes induced by 2% alcohol were too severe to be recovered by resveratrol during heart formation.

Moreover, our results showed that resveratrol could alter the mRNA expression of *bmpr2b*, *hand2*, and *tbx5a*, which are critical transcription factors for vertebrate heart development, in alcohol + resveratrol groups compared to untreated groups exposed to the same alcohol concentrations.[5-7] Alteration of these genes' expression during the process of heart development may lead to its disturbance. Our work demonstrated that the cardiovascular protective effects of resveratrol might be mediated *via* altering the expression of heart-related genes.

In summary, resveratrol can recover morphologic and functional abnormalities induced by alcohol to a certain extent. Resveratrol exhibited more significant protective effects against heart deformations induced by 1% alcohol compared to those induced by 2% alcohol, which means that a higher alcohol concentration can induce more severe damage that cannot be alleviated by resveratrol afterwards. Analysis of heart-related gene expression provides evidence for resveratrol's protective effects against alcohol-induced heart abnormalities. Results of this study also provide a better understanding for the protective effects of resveratrol against FASD.

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Conflicts of interest

None.

References

1. Burd L, Deal E, Rios R, Adickes E, Wynne J, Klug MG. Congenital heart defects and fetal alcohol spectrum disorders. Congenit Heart Dis 2007;2:250–255. doi: 10.1111/j.1747-0803.2007.00105.x.

2. Raj P, Louis XL, Thandapilly SJ, Movahed A, Zieroth S, Netticadan T. Potential of resveratrol in the treatment of heart failure. Life Sci 2014;95:63–71. doi: 10.1016/j.lfs.2013.12.011.
3. Bilotta J, Barnett JA, Hancock L, Saszik S. Ethanol exposure alters zebrafish development: a novel model of fetal alcohol syndrome. Neurotoxicol Teratol 2004;26:737–743. doi: 10.1016/j.ntt.2004.06.011.
4. Ramlan NF, Sata N, Hassan SN, Bakar NA, Ahmad S, Zulkifli SZ, *et al*. Time dependent effect of chronic embryonic exposure to ethanol on zebrafish: morphology, biochemical and anxiety alterations. Behav Brain Res 2017;332:40–49. doi: 10.1016/j.bbr.2017.05.048.
5. Tsuchihashi T, Maeda J, Shin CH, Ivey KN, Black BL, Olson EN, *et al*. Hand2 function in second heart field progenitors is essential for cardiogenesis. Dev Biol 2011;351:62–69. doi: 10.1016/j.ydbio.2010.12.023.
6. Monteiro R, van Dinther M, Bakkers J, Wilkinson R, Patient R, ten Dijke P, *et al*. Two novel type II receptors mediate BMP signalling and are required to establish left-right asymmetry in zebrafish. Dev Biol 2008;315:55–71. doi: 10.1016/j.ydbio.2007.11.038.
7. Musso G, Mosimann C, Panakova D, Burger A, Zhou Y, Zon LI, *et al*. Generating and evaluating a ranked candidate gene list for potential vertebrate heart field regulators. Genom Data 2015;6:199–201. doi: 10.1016/j.gdata.2015.09.015.

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