

Isoniazid causes heart looping disorder of zebrafish embryo by inducing oxidative stress

Hongye WANG (✉ 771647652@qq.com)

Nanjing Medical University <https://orcid.org/0000-0002-6467-0316>

Liu Yihai

nanjing medical university

Wei Xiyi

nanjing medical university

cheng cao

Nanjing Medical University

Hu Tingting

nanjing medical university

Research article

Keywords: Isoniazid, heart looping disorder, oxidative stress

Posted Date: September 16th, 2019

DOI: <https://doi.org/10.21203/rs.2.14469/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at BMC Pharmacology and Toxicology on March 12th, 2020. See the published version at <https://doi.org/10.1186/s40360-020-0399-2>.

Abstract

The cardiotoxicity of isoniazid on zebrafish embryos and its underlying mechanism remained unclear. Here, we exposed zebrafish embryos at 4 hours post fertilization to different levels of isoniazid and recorded the morphology and number of malformed and dead embryos under the microscope. The high concentration of isoniazid group showed more malformed and dead embryos compared with low dose of isoniazid group and control group. Besides, the morphology of heart and its alteration were visualized using the transgenic zebrafish (*cmlc2: GFP*) and confirmed by in situ hybridization. The negative effects of isoniazid on the developing heart were characterized by lower heart rate and more heart looping disorders. Mechanistically, PCR showed decreased expression of heart-specific transcription factors exposed to isoniazid. Oxidative stress was induced by Isoniazid in cardiomyocytes, mediated by decreased activity of CAT and SOD, which could be rescued by ROS scavenger. In conclusion, we demonstrated that isoniazid lead to heart looping disturbance by downregulating cardiac specific transcription factors and inducing cardiomyocytes apoptosis.

Introduction

Tuberculosis is a highly infectious disease which has a high prevalence in China(1). The common anti-tuberculosis drugs include isoniazid, rifampicin, ethambutanol, pyrazinamide, of which isoniazid is regarded as an irreplaceable first-line anti-tuberculosis drug(2). Isoniazid, also known as isonicotinyldrazide (INH), was first introduced in 1953 and still used today for its high efficacy against tubercle bacillus(3). Isoniazid inhibited the growth and reproduction of tubercle bacillus by producing reactive oxygen species and reactive nitrogen species(4). What's more, isoniazid destroys bacterium cell walls by preventing from the synthesis of branching acids, mediated by activation of KatG upon its entry into tubercle bacillus, whose mutation was associated with the resistance to isoniazid of tubercle bacillus(4, 5).

In addition to the extremely strong anti-tuberculosis effect, the side effects of isoniazid were also observed clinically(6). It has been reported that the common side effect is liver damage, as evidence by about 20% of the patients with increased AST and ALT in blood after administration of isoniazid(7), and about 1% patients showing severe hepatocyte damages(8). Meanwhile, a few studies also found that isoniazid could lead to skin rash reaction(9) and endocrine disturbance(10). Although Isoniazid is regarded as pregnant class C drugs by Food and Drug Administration (11), some studies had pointed out the potential toxic effects to fetal by the isoniazid. Zhang et al found that isoniazid affected the liver development of zebrafish embryos and caused liver injury by constructing *fabp10a-mcherry* transgenic strain zebrafish(12). And the abnormal development of nervous system, skull bone and cartilage zebrafish embryo due to isoniazid was also demonstrated in vitro and in vivo(13). However, there is no definitive report regarding whether isoniazid has toxic effects on developing heart of zebrafish.

According to epidemiological statistics, congenital heart disease was about 3–4% in infants(14–16), which became one of the most common cause of neonatal death(17). The types of congenital heart

disease include ventricular septal defect, atrioventricular valve defect, transposition of aorta, and constriction of aorta. The occurrence of congenital heart disease is attributed to many factors, such as genetic factors, environmental factors, pathogenic microorganism infections, and drug toxicity, among which the drug toxicity played an important role in cardiac development defects(18). For example, tilmicosin(19), doxorubicin(20), cisplatin(21) and diclofenac sodium(21) have been previously reported to induce cardiotoxicity by regulating oxidative stress. However, the teratogenicity and cardiotoxicity of isoniazid on fetal heart development was still rarely reported. Zebrafish is a widespread kind of model animal owing to short spawning periods and transparent embryos, which is often used to investigate drug toxicity and molecular mechanism of development (22, 23). In our study, utilizing a kind of Cmlc2-GFP heart transgenic zebrafish, we observed that isoniazid disturbed the cyclization process during heart development of zebrafish embryos and caused congenital cardiac malformation, which was mediated by inducing embryo oxidative stress and cardiomyocytes apoptosis.

Materials And Methods

2.1 zebrafish husbandry

The wide type zebrafish (AB strain) brought from the institute of Hydrobiology of Chinese Academy of Science (Wuhan, China) and transgenic zebrafish (AB Cmlc2-GFP) given by Dr. Lou in Nanjing University animal model Institute, were kept in the animal model center of key laboratory of Jiangsu province human functional genomics. A $28 \pm 0.5^{\circ}\text{C}$ temperature of recycled water and a 14h light cycle per day were set to mimic the circadian rhythm. Zebrafish were fed with brine shrimp twice a day. At 17:00 o'clock, we transferred the male and female adult zebrafish into the mating tank with a ratio of 2:1 and segregate them with a partition. In the next morning, we moved the partition at 9:00 o'clock and the mating process lasted for 2h. After spawning, the embryos were collected in clean fish water. The experiment protocols were approved by the animal ethic committee of Nanjing Medical University.

2.2 Chemical exposure experiment

We chose a dose of (0mM, 0.01mM, 0.04mM, 0.16mM, 0.64mM, 2.56mM, 10.24mM) isoniazid (I3377–50G;Sigma) for the dose-effect assay according to the therapeutic range of 3~5 $\mu\text{g}/\text{ml}$ isoniazid(24). The N-acetyl-L-cysteine (NAC; A7250–50G;Sigma) were dissolved in clean embryo culture water for 1mM. After 4 hours post fertilization (hpf) we selected 80 well-grown embryos for each group under the microscope. The culture water containing isoniazid was changed every 12 hours per day and these embryos were kept in the incubator under 28°C and 5% CO_2 . We recorded the number and morphology of malformed and dead embryos, and then removed dead embryo every 12h after exposure. Each chemical exposure treatment was performed in triplicate.

2.3 Morphology observation

We observed the morphology of embryos in different developing stage (24hpf, 48hpf, 72hpf) under the white light with a fluorescent microscope (Nikon, Tokyo, Japan). In each stage, the total number of dead,

unhatched and malformed embryos was recorded. We randomly selected 20 larvae and recorded the heart rate within one minute in each experimental group. A transgenic zebrafish strain (Cmlc2-GFP), where a green fluorescent protein gene was inserted under the promoter of a cardiomyocyte-specific gene of Cmlc2, was applied to observe morphology changing of heart development. All the experiments were done in three times.

2.4 Real-time PCR

After 48 hours chemical exposure, the total RNA of 20 larvae were isolated by Trizol reagent. The total RNA in each group were reverse transcribed for cDNA with TaKaRa RT reagent kit. The primers of cardiomyocyte-specific genes were synthesized by Invitrogen (Shanghai, China) and their sequences were listed in Table1. All real-time PCR was performed in triplicate after the mixture of SYBR green, primers, cDNA, and RNase-free water in an appropriate ratio. A house keeping gene 18s ribosomal RNA was used for normalization.

2.5 Acridine orange staining

Acridine orange, a permeable nucleic acid-selective fluorescent dye (Thermo Fisher Scientific) was used to assess the apoptosis level of cardiomyocytes during the development of zebrafish exposed to different doses of isoniazid. Ten randomly selected embryos at 48 hpf were stained with AO for 30min and then washed by PBS. A fluorescence microscope (Nikon, Tokyo, Japan) was used for photography.

2.6 ROS detection

2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA; Beyotime Biotechnology, China), a permeable and sensitive probe, was used for detecting reactive oxygen species in this study. We randomly selected 10 embryos at 48 hpf, and stained them for 40 minutes with 100 μ M DCFH-DA diluted in PBS. After washed out with PBS for 10 minutes, all the groups were photographed under a fluorescence microscope under the FITC channel. All the steps are finished in the absence of light.

2.7 Anti-oxidant enzyme activity assay

In each groups, 20 zebrafish embryos were lysed in RIPA and Cocktails. Commercial kits (Beyotime Biotechnology, China) were used to detect the activity of two important anti-oxidant enzyme, namely superoxide dismutase (SOD) and catalase (CAT). Besides, we also evaluated the level of malondialdehyde (MDA), another marker for oxidative stress. All the experiments were performed in three times.

2.8 In situ hybridization

Embryos for situ hybridization were exposed to propylenethiourea (PTU), a chemical reagent to remove the melanin of larvae. After that, we fixed the embryos in 4% Paraformaldehyde and preserved in 4°C. The experiment of in situ hybridization lasted for 3 days. In the first day, we used ethanol in concentration of

25%, 50%, 75%, 100% for dehydration, and a protease kinase to digest the tissues for 10 minutes. Next, triethanolamine was added to acidized them and the nuclear acid probe of *cmlc2* for hybridization under the temperature of 60°C. In the second day, we used 2x saline sodium citrate (SSC) and 0.2x SSC buffer respectively to wash out the residual probes. Then we added the antibody against digoxin overnight under the temperature of 4°C. In the last day, we washed out the antibody with 1% maleic acid buffer (MAB) and dried the embryos with BM purple for 10 minutes. Finally, we observed these dried embryos in a plate with 5% Agar powder with upright microscope (Olympus, Japan) under the white light.

2.9 Statistical analysis

Continuous variables were presented as mean and SD, while categorical variables were presented as number and percentage. For continuous variables, the difference between all groups was compared with one-way ANOVA test. And chi-square test was used for categorical variables. $P < 0.05$ was deemed as a significant statistical difference. The asterisk denoted statistical significance between two groups (* $P < 0.05$, ** $P < 0.1$, *** $P < 0.001$). All statistical tests were finished in SPSS 22.0.

Results

1. The effect of isoniazid on mortality and hatchability of embryos

In our experiment, the mortality and hatching rate of zebrafish embryos exposed to different concentrations of isoniazid (0mM, 0.01mM, 0.04mM, 0.16mM, 0.64mM, 2.56mM, 10.24mM) at different time points were recorded (Figure 1B, C). Compared with the unexposed control group, the mortality of embryos in the treated group increased, and the hatchability decreased, which both were associated with dose. Also, we observed that zebrafish embryos had obviously abnormal development of somatic ganglia and yolk sac edema at 48 and 72hpf regardless of no difference in morphology at the early stage (Figure 1A).

2. The effect of isoniazid on heart development of embryos

The heart development of zebrafish embryos underwent a series process including cardiogenic specification and differentiation, formation of bilateral heart field, myocardia tube rotation, cardiac looping and chamber ballooning and atrioventricular valves formation(25). We observed the cardiac morphological changes at 48hpf by using *Cmlc2*-GFP transgenic zebrafish. Compared with control group, treatment groups both developed cardiac cyclization disorders during 48hpf, which could be alleviated by anti-oxidative NAC. (Figure 2A). Specifically, the results of in situ hybridization of *cmlc2*, also indicated that isoniazid caused cardiac cyclization disorders (Figure 2B). Some of these embryos appeared yolk sac edema, of which the proportion in treatment groups was higher than that in control group (Figure2C). Besides, we found that isoniazid could decrease the heart rate of zebrafish larve, which could be rescued by NAC treatment (Figure 3). At the same time, it was found that severe arrhythmia appeared in the 0.04mM and 0.16mM group (Data not shown). A number of key transcriptional factors including *Tbx5*, *Gata4*, *Hand2*, which control the cardiac cyclization during the cardiac development, showed a decreased

expression in embryos treated with isoniazid (Figure 4). Also did some marker genes associated with ventricular and atrial development, such as *Amhc*, *Vmhc* and *Cmlc2* (Figure 4). These experimental results collectively suggested that isoniazid would cause embryo development deficiency, which may be mediated by inhibiting heart looping.

3. Oxidative stress in cardiomyocytes induced by isoniazid

Previous studies have reported that isoniazid may cause the accumulation of reactive oxygen species in cells, and high ROS can cause heart looping deficiency during cardiac development. In order to explore the mechanism underneath heart looping defects, we used ROS fluorescence probe to label 48hpf zebrafish embryos and did MDA assay. It was demonstrated that there was a serious oxidation stress in the heart of zebrafish embryos in the treatment groups, and the effect was linked to isoniazid concentration (Figure 5A, D). Further, we detected the activity of two important anti-oxidant enzymes, SOD and CAT, and consequently, both of these two enzymes had a lower activity in the 0.16mM treated group (Figure 5B, C). However, a higher activity of SOD enzyme was found in 0.04mM treated group, indicating the potential compensatory effects in the embryos for the scavenge of ROS (Figure 5B). Next, an anti-oxidant chemical was used to do the rescue experiment, hopefully, we found that NAC can reverse the heart development deficiency caused by isoniazid effectively (Figure 2A, B, C, 3, 4). These results may explain that isoniazid cause heart looping deficiency depending on oxidative stress. Finally, we found that isoniazid induced cardiac cells apoptosis by AO staining and ROS scavenger can rescue the apoptosis (Figure 5E).

Discussion

As a first-line anti-tuberculosis drug, Isoniazid plays an irreplaceable role in the treatment of tuberculosis(2). Liver toxicity and gastrointestinal reaction were predominant side effects found during clinical drug use, and several cases indicates that isoniazid can cause potential cardiotoxicity. In our study, we found that isoniazid can cause deficiency of cardiac development through a ROS dependent manner. From our results, high level Isoniazid reduced the hatch and survival rate of zebrafish embryos, which was linked to heart development using heart-specific transgenic zebrafish. In combined with ROS and AO staining, and oxidant enzymes and antioxidant enzymes assays, Isoniazid could induce oxidative stress and cardiomyocyte apoptosis during heart development. It has been reported that isoniazid can cause oxidative stress and apoptosis in tumor cell line Hep3B, which may be related to its high metabolic demand and its strong proliferative ability. So the differential response to Isoniazid between neonatal myocytes and adult cardiomyocytes was accounted for that embryonic cells owned a strong proliferative capacity while mature cardiac cells had a more mature antioxidant system.

During embryonic development, immature cells are stem-like and have strong ability of proliferation and differentiation. Therefore, they are prone to external environment and drugs for affecting transcription regulation. The heart is the first organ to form during the embryonic development, including the following stages: the formation of cardiogenic zones from cardiac progenitor cells, with the first cardiogenic zone

and the second cardiogenic zone(26, 27); the appearance of a linear cardiac tube due to separation and migration of cardiogenic zones at proto-intestinal stage, with heart starting beating; the looping of the linear cardiac tube; the arising of primitive atrium and ventricle structure from endocardial endothelial cells, with undergoing endothelial mesenchymal transformation into endocardial cushions and an ultimate heart came into being after cardiac cells remodeling(28–30). Heart development was regulated by a combination of transcription factors, whose down-regulation lead to defects in heart development, teratogenicity and even death of embryos(31). In our experiment, transcription factors related to cardiac development was down-regulated in the high concentration group, as explained the cardiotoxicity of isoniazid from the molecular level.

ROS is a series of chemicals with strong oxidative capacity produced during cell metabolism, including superoxide radicals, hydroxyl radicals and hydrogen peroxides. High concentration of ROS induced oxidative stress and mitochondrial dysregulation in cells, causing the oxidation of lipids, proteins and nucleic acids and destroying their biological functions. ROS is a signal molecule and plays an important part in the process of embryonic development. It participated in the development of the nervous system, promoting the differentiation and the formation of the nerve, and the development of lung and vascular(32). Recently, an article reported that high levels of ROS lead to heart looping disorder during heart development(33, 34). There were also many reports that environmental poisons and drugs such as doxorubicin(35) and chlorpyrifos(36) caused abnormal development of various organs by inducing ROS accumulation. In our study, we found similar consequences that ROS was highly concentrated in the heart and cardiac looping was impaired in the treatment group, suggesting that isoniazid caused cardiac dysplasia by upregulating ROS. In the high concentration group, the atrial and ventricle became smaller than that in the middle concentration group and the control group, which may be due to the apoptosis and cell cycle arrest of developing cardiomyocytes caused by ROS. However, the molecular mechanism and signaling pathway underlying the cardiotoxicity of isoniazid remains to be further investigated.

Declarations

Acknowledgements

Not applicable.

Author contribution statement

Wang hongye, Liu yihai and Wei xiyi contributed equally to this work. All the experiment were done by Wang hongye and Liu yihai. Wei xiyi was in charge of data analysis and technical graphics. Cao cheng and dr Hu tingting helped revised this manuscript.

Data availability statement.

All data generated or analyzed during this study are included in this article. All data in this research is available for this journal.

Competing interests

The authors declared that they have no competing interests.

References

1. Wang L, Zhang H, Ruan Y, et al.: Tuberculosis prevalence in China, 1990–2010; a longitudinal analysis of national survey data. *Lancet* 383: 2057–2064, 2014.
2. Schito M, Migliori GB, Fletcher HA, et al.: Perspectives on Advances in Tuberculosis Diagnostics, Drugs, and Vaccines. *Clin Infect Dis* 61Suppl 3: S102–118, 2015.
3. Sarich TC, Zhou T, Adams SP, Bain AI, Wall RA and Wright JM: A model of isoniazid-induced hepatotoxicity in rabbits. *J Pharmacol Toxicol Methods* 34: 109–116, 1995.
4. Timmins GS, Master S, Rusnak F and Deretic V: Nitric oxide generated from isoniazid activation by KatG: source of nitric oxide and activity against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 48: 3006–3009, 2004.
5. Suarez J, Ranguelova K, Jarzecki AA, et al.: An Oxyferrous Heme/Protein-based Radical Intermediate Is Catalytically Competent in the Catalase Reaction of *Mycobacterium tuberculosis* Catalase-Peroxidase (KatG). *Journal of Biological Chemistry* 284: 7017–7029, 2009.
6. Nahid P, Dorman SE, Alipanah N, et al.: Executive Summary: Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: Treatment of Drug-Susceptible Tuberculosis. *Clinical Infectious Diseases* 63: e147, 2016.
7. JR M, HJ Z, KG I, et al.: Isoniazid liver injury: clinical spectrum, pathology, and probable pathogenesis. *Annals of Internal Medicine* 84: 181, 1976.
8. Ahmad J, Bach N, Bansal M, et al.: Features and Outcomes of 899 Patients With Drug-Induced Liver Injury: The DILIN Prospective Study. *Gastroenterology* 148: 1340–1352.e1347, 2015.
9. Yashika G, Rajeshwari G, Sourabh J and Arun K: A rare case of isoniazid-induced erythroderma. *Indian Journal of Pharmacology* 47: 682–684, 2015.
10. Li F, Wang P, Liu K, et al.: A high dose of isoniazid disturbs endobiotic homeostasis in mouse liver. *Drug Metabolism & Disposition* 44: 1742, 2016.
11. Mathad JS and Gupta A: Tuberculosis in pregnant and postpartum women: epidemiology, management, and research gaps. *Clin Infect Dis* 55: 1532–1549, 2012.
12. Zou Y, Zhang Y, Han LW, et al.: Impact of isoniazid on developmental toxicity and behavior of zebrafish larvae. *Shandong Science* 2016.

13. Strecker R, Weigt S and Braunbeck T: Cartilage and bone malformations in the head of zebrafish (*Danio rerio*) embryos following exposure to disulfiram and acetic acid hydrazide. *Toxicology & Applied Pharmacology* 268: 221–231, 2013.
14. Bjornard K, Riehle-Colarusso T, Gilboa SM and Correa A: Patterns of congenital heart defects, Metropolitan Atlanta, 1978 to 2005. *Birth Defects Research Part A Clinical & Molecular Teratology* 97: 87–94, 2013.
15. Moons P, Sluysmans TW, D, Massin M, Suys B, Benatar A and Gewillig M: Congenital heart disease in 111 225 births in Belgium: birth prevalence, treatment and survival in the 21st century. *Acta Pædiatrica* 98: 472–477, 2009.
16. Shah GS, Singh MK, Pandey TR, Kalakheti BK and Bhandari GP: Incidence of congenital heart disease in tertiary care hospital. *Kathmandu University Medical Journal* 6: 33–36, 2008.
17. Sanchez-Castro M, Pichon O, Briand A, et al.: Disruption of the SEMA3D Gene in a Patient with Congenital Heart Defects. *Human Mutation* 36: 30–33, 2015.
18. Källén BA and Otterblad OP: Maternal drug use in early pregnancy and infant cardiovascular defect. *Reproductive Toxicology* 17: 255–261, 2003.
19. Ibrahim AE and Abdel-Daim MM: Modulating Effects of *Spirulina platensis* against Tilmicosin-Induced Cardiotoxicity in Mice. *Cell journal* 17: 137–144, 2015.
20. Abushouk AI, Ismail A, Salem AMA, Afifi AM and Abdel-Daim MM: Cardioprotective mechanisms of phytochemicals against doxorubicin-induced cardiotoxicity. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 90: 935–946, 2017.
21. Abdellatif SA, Galal AA, Farouk SM and Abdel-Daim MM: Ameliorative effect of parsley oil on cisplatin-induced hepato-cardiotoxicity: A biochemical, histopathological, and immunohistochemical study. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 86: 482–491, 2017.
22. Schwerte T and Pelster B: Digital motion analysis as a tool for analysing the shape and performance of the circulatory system in transparent animals. *Journal of Experimental Biology* 203: 1659–1669, 2000.
23. Development: Test No.203: Fish, Acute Toxicity Test. *Oecd Guidelines for the Testing of Chemicals* volume 1: 1–10(10), 1992.
24. Shah I, Jadhao N, Mali N, Deshpande S, Gogtay N and Thatte U: Pharmacokinetics of isoniazid in Indian children with tuberculosis on daily treatment. *The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease* 23: 52–57, 2019.

25. Bakkers J: Zebrafish as a model to study cardiac development and human cardiac disease. *Cardiovasc Res* 91: 279–288, 2011.
26. Buckingham M, Meilhac S and Zaffran S: Building the mammalian heart from two sources of myocardial cells. *Nat Rev Genet* 6: 826–835, 2005.
27. Vincent SD and Buckingham ME: How to make a heart: the origin and regulation of cardiac progenitor cells. *Current topics in developmental biology* 90: 1–41, 2010.
28. Bruneau BG: Signaling and transcriptional networks in heart development and regeneration. *Cold Spring Harb Perspect Biol* 5: a008292, 2013.
29. Olson EN: Gene regulatory networks in the evolution and development of the heart. *Science* 313: 1922–1927, 2006.
30. Waardenberg AJ, Ramialison M, Bouveret R and Harvey RP: Genetic networks governing heart development. *Cold Spring Harb Perspect Med* 4: a013839, 2014.
31. Bruneau BG: The developmental genetics of congenital heart disease. *Nature* 451: 943–948, 2008.
32. Olguin-Albuerne M and Moran J: ROS produced by NOX2 control in vitro development of cerebellar granule neurons development. *ASN Neuro* 72015.
33. Daugaard M, Nitsch R, Razaghi B, et al.: Hace1 controls ROS generation of vertebrate Rac1-dependent NADPH oxidase complexes. *Nat Commun* 4: 2180, 2013.
34. Razaghi B, Steele SL, Prykhozhij SV, et al.: hace1 Influences zebrafish cardiac development via ROS-dependent mechanisms. *Dev Dyn* 247: 289–303, 2018.
35. Zennaro C, Mariotti M, Carraro M, et al.: Podocyte developmental defects caused by adriamycin in zebrafish embryos and larvae: a novel model of glomerular damage. *PLoS One* 9: e98131, 2014.
36. Jin Y, Liu Z, Peng T and Fu Z: The toxicity of chlorpyrifos on the early life stage of zebrafish: a survey on the endpoints at development, locomotor behavior, oxidative stress and immunotoxicity. *Fish Shellfish Immunol* 43: 405–414, 2015.

Tables

Table1

Sequences of Primers in RT-PCR

| Target Gene | Primer Sequences |
|-------------|---|
| 18s | Forward:5'TCGCTAGTTGGCATCGTTTATG 3' Reverse:5' CGGAGGTTCGAAGACGATCA 3' |
| Amhc | Forward:5' AAGGTAAAATCCTACAAACGTTCCGG 3' Reverse:5' CAAACAAATCAAAGTGCGATTGCAC 3' |
| Vmhc | Forward:5' ACATAGCCCGTCTTCAGGATTTGG 3' Reverse:5' GAGAGAAAGGCAAGCAAGTACTGG 3' |
| Cmlc2 | Forward:5' AGACCCAGAGGAAACCATCC 3' Reverse:5' TTGGGTCATTAGCAGCCTCT 3' |
| Tbx5 | Forward:5'CGCTATAAATTCGCCGATAACAA 3' Reverse:5' AGACACCAGTTGCCTCATCCAG3' |
| Hand2 | Forward:5' GGACATTCTGGACAAAGATGAA 3' Reverse:5' GCCAACCAGTTCTCCCTTTA 3' |
| Gata4 | Forward:5' CCAGACACACACCAGCTCTACAC 3' Reverse:5' ATCAGGCTGTTCCACACTTCACT 3' |

Figures

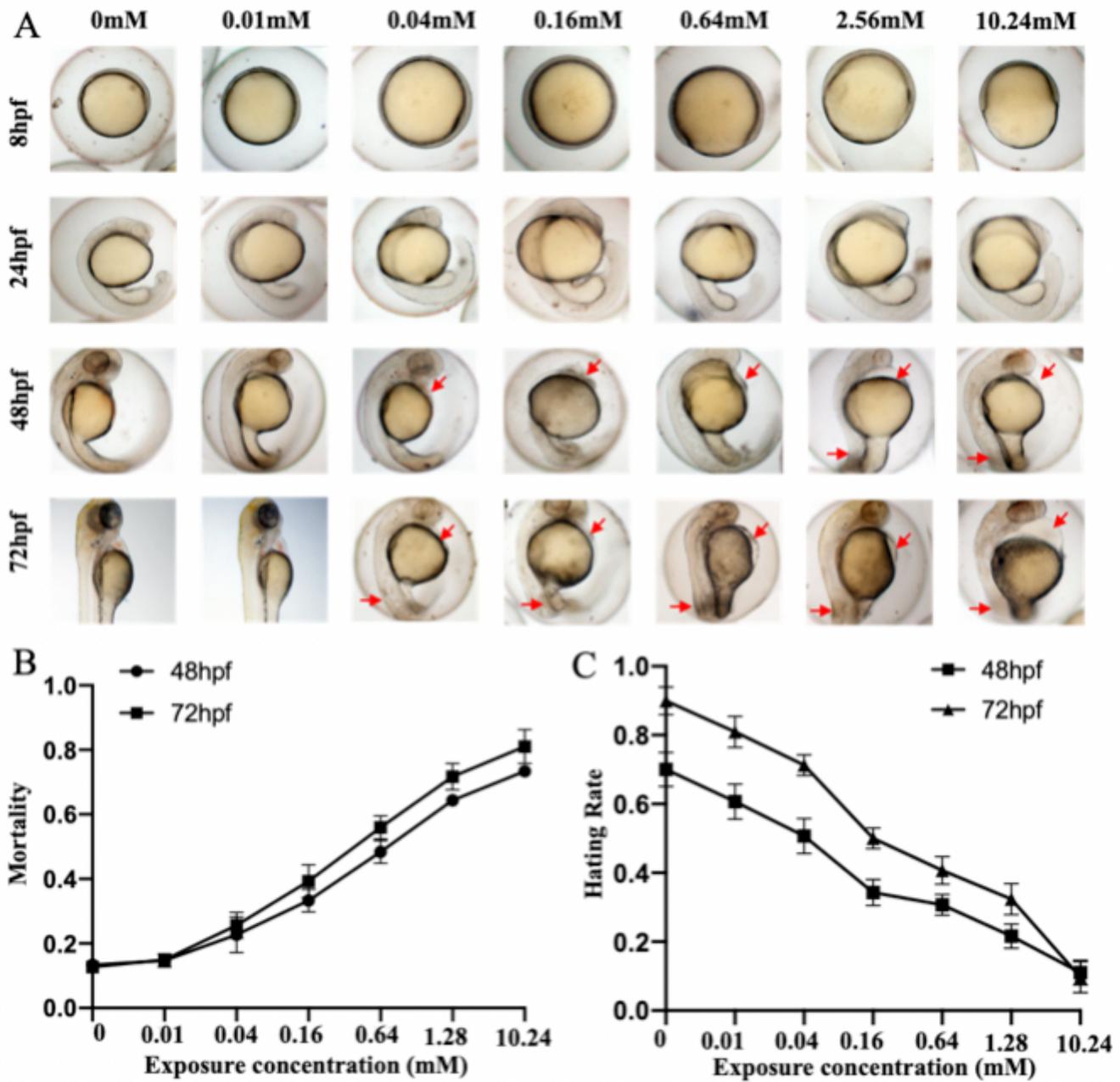


Figure 1

Morphology alteration(A), mortality(B) and Hating rate (C) of zebrafish embryos exposed to different concentrations of isoniazid at 48 and 72hpf. * $P < 0.05$, ** $P < 0.1$, *** $P < 0.001$.

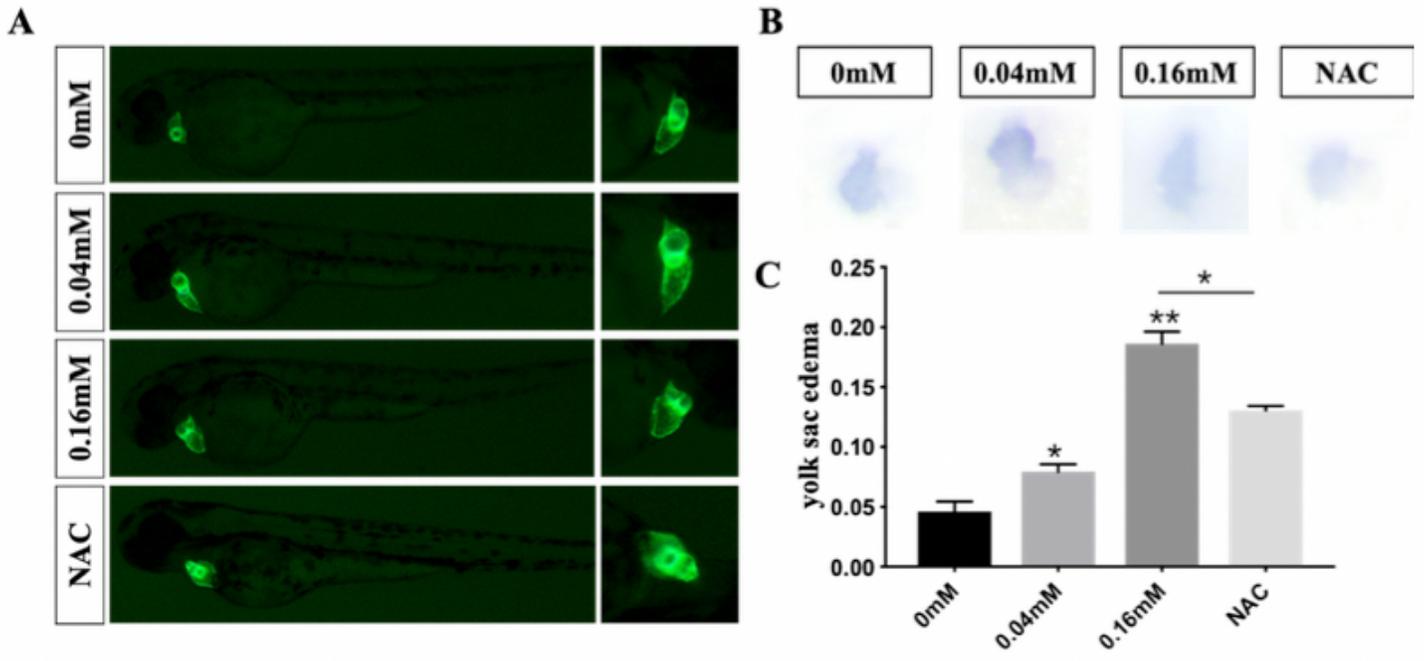


Figure 2

The alteration in the cardiac morphology at 48hpf by using transgenic strain zebrafish (Cmlc2-GFP) (A). The results of in situ hybridization by using a *cmlc2* probe (B). Isoniazid lead to yolk sac edema during the cardiac development (C).

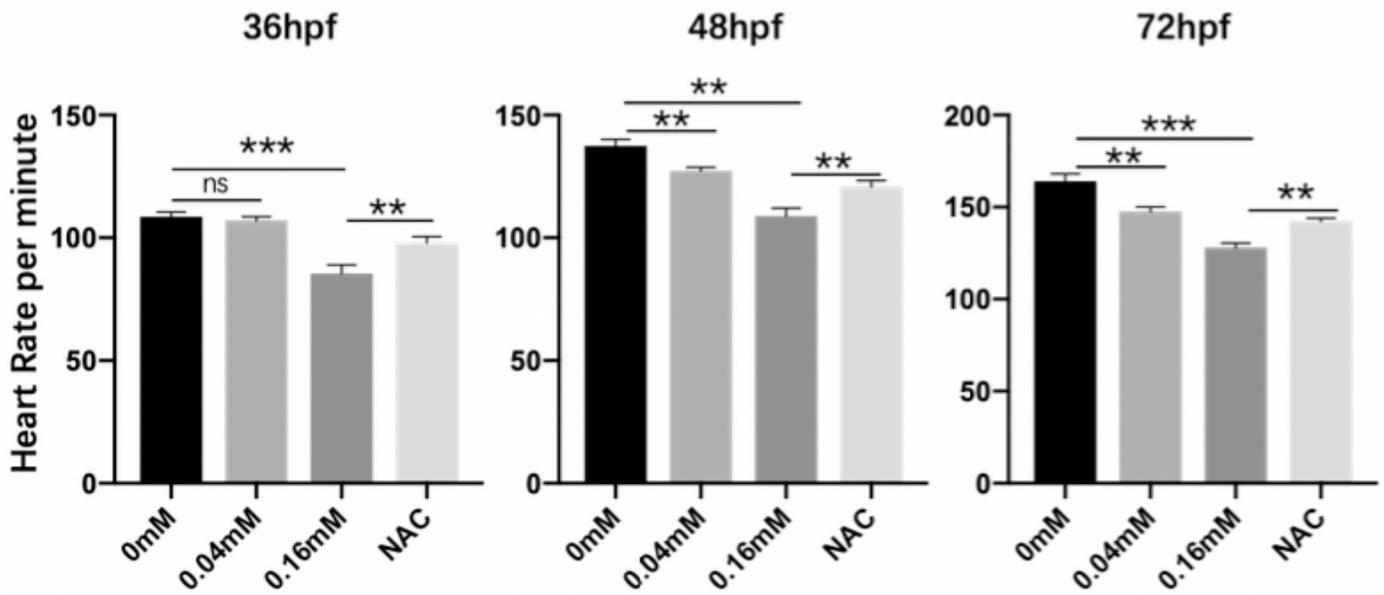


Figure 3

The heart rate of zebrafish embryos recorded at different times in control, 0.04mM, 0.16mM and NAC groups.

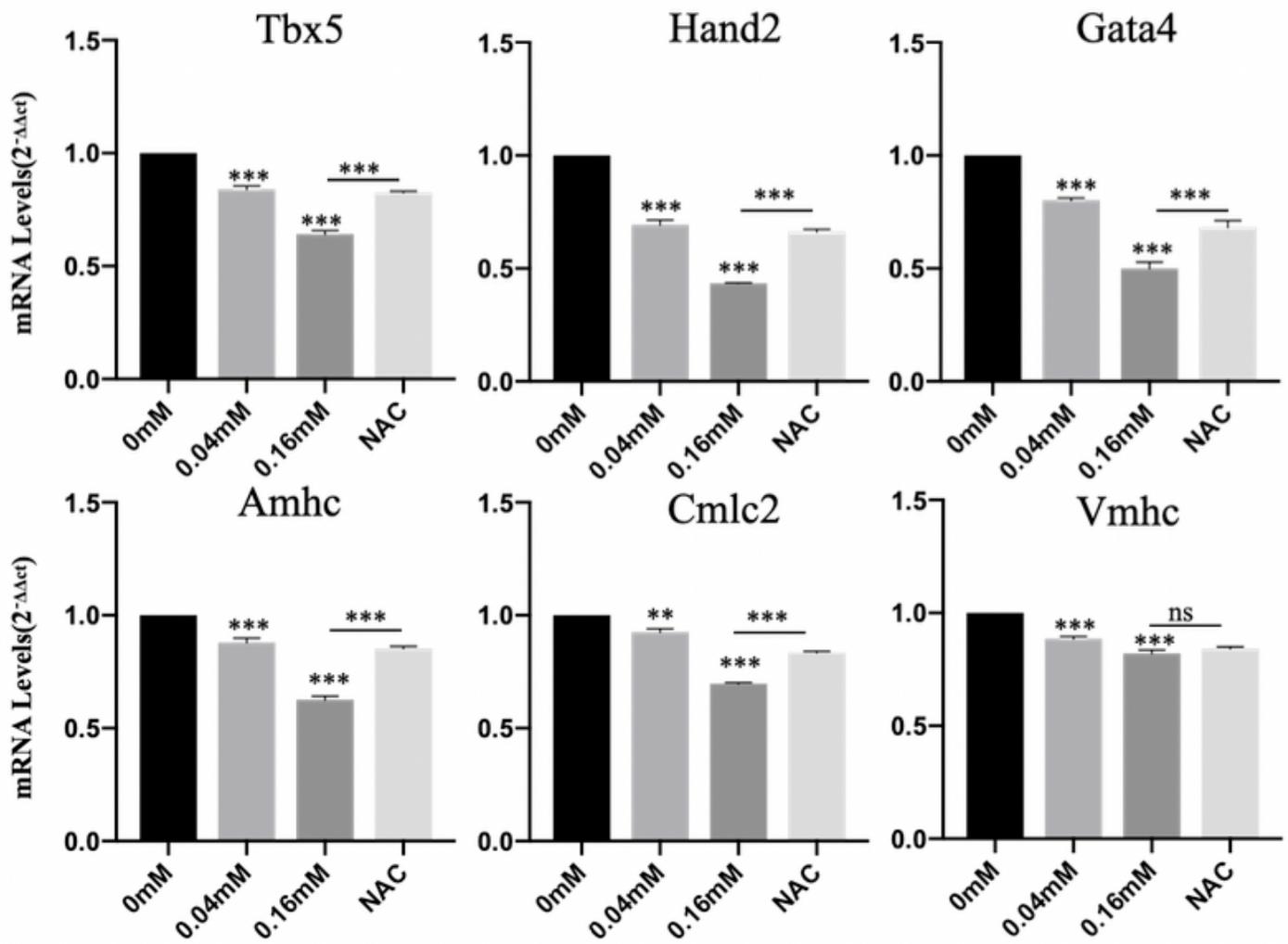


Figure 4

The expression of four cardiac-specific transcript factors in zebrafish embryos after 48 hpf of isoniazid exposure.

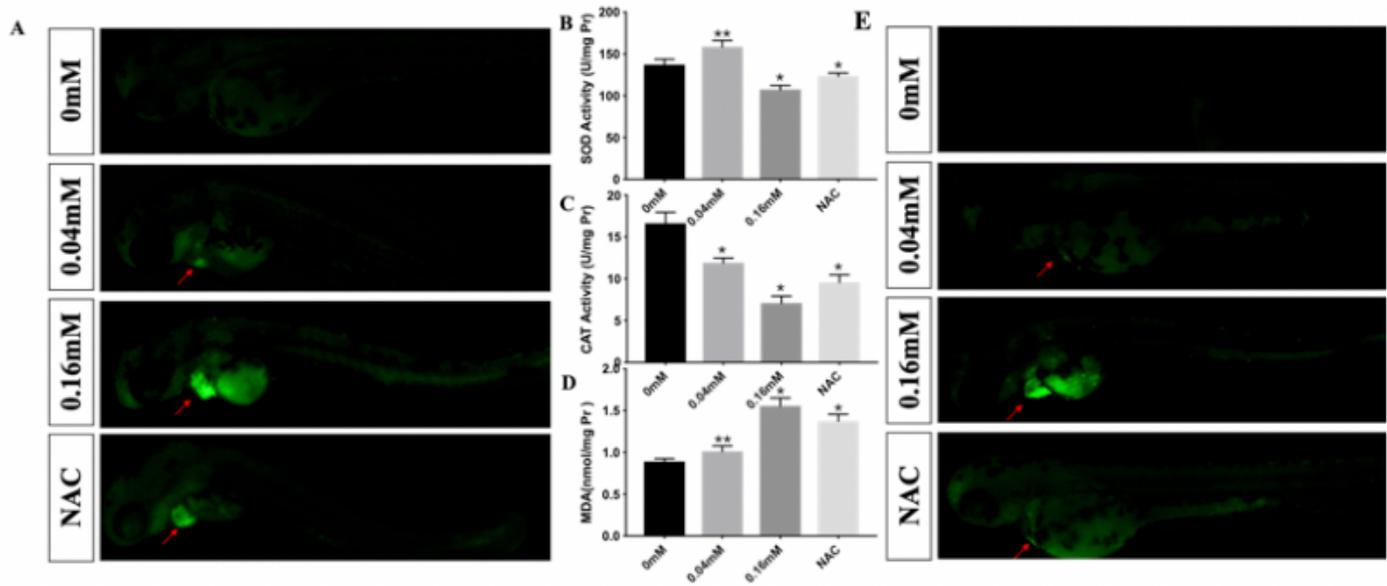


Figure 5

The ROS level in control, 0.04mM, 0.16mM and NAC groups (A). The activity of CAT (B) and SOD (C) induced by Isoniazid. The concentration of MDA (D) and apoptosis level (E) in different treated groups. The unit of SOD and CAT were U/mg protein while MDA nmol/mg protein.