Use of Coronary Ultrasound Imaging to Evaluate Ventricular Function in Adult Zebrafish

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Abstract

So far, imaging of the adult zebrafish heart and assessment of heart failure in adult zebrafish have been very limited. Here, we describe a new method for *in vivo* imaging of the hypertrabeculated heart of the adult zebrafish using miniaturized cardiac ultrasound catheters obtained from the cardiac catheterization laboratory. This method allows the observation of the ventricle of zebrafish and the assessment of ventricular diameters during diastole and systole, as well as heart rate and fractional shortening. Significant changes in these parameters were detected through the use of an adult zebrafish heart failure model induced by chronic anemia. This imaging technique opens the door to detailed *in vivo* analysis of the adult heart failure phenotype in zebrafish.

Keywords: cardiac function, miniaturized ultrasound system, heart failure, adult zebrafish

Introduction

OVER THE PAST DECADE, the zebrafish has become an increasingly popular vertebrate model to study heart development and heart disease.¹ The transparency of zebrafish embryos allows observation of the heart and vasculature so that heart morphology and function can be easily analyzed. In addition, the ease of genetic manipulation makes them an incredible tool to identify novel cardiovascular disease genes and decipher pathways leading to congenital heart disease. Zebrafish larvae develop rapidly and have the ability to survive without a functional cardiovascular system during the first week of development. Zebrafish embryos also offer the opportunity to test a variety of molecules through high throughput screening to facilitate the development of new therapies.^{2,3}

Although zebrafish embryos are outstanding as an *in vivo* model and have unique genetic tools, many human cardiovascular diseases occur at adulthood with a progressive pathogenesis. As such, the adult zebrafish model has been studied for its ability to model human cardiovascular diseases. Several cardiomyopathy models have been described in adult zebrafish. A chronic anemia-inducing hypertrophy phenotype was obtained either by repeated treatments of the adult fish with phenylhydrazine hydrochloride (PHZ)⁴ or through the use of a mutant fish including a Band 3 mutation in erythrocytes.⁵ In another model, cardiomyopathies were induced by intraperitoneal injection of doxorubicin, an anthracycline drug used for cancer treatment.⁶ However, the number of cardiovascular research groups working on adult zebrafish is still limited and the main focus has been on cardiac regeneration after manual

cardiac injury^{7,8} or genetic cardiomyocyte ablation.⁹ Indeed, the measurement of cardiac function in nontranslucent adult zebrafish is a challenge and up to now has limited the use of adult zebrafish as a model of cardiovascular disease.

Here we present a protocol to analyze ventricular characteristics in adult zebrafish using a miniaturized ultrasound system. This protocol was used to investigate the cardiac phenotype in an adult cardiomyopathic zebrafish model.

Materials and Methods

Zebrafish and cardiomyopathy model

All animal experiments were conducted in accordance with the regulations of the Animal Welfare Act of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and the protocol was approved by a local Ethics Committee and by the regional Veterinary Department. Eight to 10-month-old male wild-type AB zebrafish were used in this study. Before starting an experiment, fish from the same breeding stock were weighed before being homogeneously distributed into experimental tanks maintained at 28°C, in a 14:10-h light–dark cycle. Animals were fed to satiety twice daily with dry food. After 1 week of acclimation, the experimental protocol started following an adapted protocol of Sun *et al.*⁴ to generate heart failure. In brief, the treated group was incubated with PHZ (Sigma-Aldrich, Bornem, Belgium) in fish water during 30 min every 3 days for 5 weeks. PHZ was used at a concentration of 1.25 µg/mL the first week followed by 2.5 µg/mL for the rest of the protocol. Treatment phases were followed by

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30 min rinsing and washing in fish water. The control group followed the same process without the addition of PHZ in the fish water.

Imaging of the zebrafish heart

A 20 MHz intravascular ultrasound (IVUS) system (Volcano s5TM IVUS imaging system; Volcano, San Diego, CA) has been used in this study to image noninvasively the ventricle of the zebrafish heart at room temperature. This system achieves a resolution inferior to 100 μ m and a beam penetration of 5 mm. Before imaging, zebrafish were anesthetized with pH-adjusted tricaine (MS-222: 160 mg/L; Sigma-Aldrich) for 5 min and placed in a supine position in a central incision of a damp sponge. The ultrasound probe (Eagle Eye Platinum ST tip catheter; Volcano) was placed on the ventral side of the fish where a beating area was seen on the fish thorax (Fig. 1A). A grayscale movie of 30 s (Supplementary Movie S1; Supplementary Data are available online at www.liebertpub.com/zeb) was recorded using DICOM Viewer 5.3.0.0 (Trillium Technology, Ann Arbor, MI). On the image (Fig. 1B), a single scan line was placed along the area of interest to observe the movement of the ventricle walls on the M-mode. On the M-mode, the greatest and smallest amplitudes were traced and were subsequently used to measure transverse end-diastole diameter (EDD) and end-systole diameter (ESD), respectively (Fig. 1C). Fractional shortening (%) was calculated using the following formula: $[(\text{EDD} - \text{ESD})/\text{EDD}] \times 100$. To measure heart rate, an investigator counted blindly the number of beats that occurred in 30 s. To obtain the number of beats per minute (bpm), this value was multiplied by 2.

At the end of the experiment, the heart was extracted from anesthetized fish. The whole heart was imaged on a Leica M165FC microscope (Lecuit Opto-Technical S.A. Howald, Luxembourg) using in-built software from Leica (LAS V4.2). The pictures were analyzed using ImageJ to determine the ventricle surface area.

Statistical analysis

Results are expressed as mean $\pm$ standard deviation. Analyses were performed with SigmaPlot v11.0 (Systat Software, San Jose, CA). Comparisons between two groups were per-

formed with two-tailed unpaired Student's *t*-test for data with a normal distribution and Mann–Whitney test for non-normally distributed data. A *p* value <0.05 was considered statistically significant.

Results

In this study, a microultrasound system was used to detect changes in ventricular size and function in adult zebrafish. A cardiomyopathy phenotype model generated by PHZ treatment was used. Ultrasound was performed to follow phenotype and motion changes during drug treatment (Fig. 2A). No significant change was observed 3 weeks after the onset of PHZ treatment. However, after 5 weeks of treatment, a significant increase of 27% in ventricle diameter was detected (Fig. 2B). To validate this increase, the hearts were excised and imaged for measurement. The ventricles from excised hearts were enlarged in PHZ-treated fish (Fig. 2C) and there was a 22% increase in ventricle surface versus control fish (Fig. 2D).

In addition, heart rate and fractional shortening were significantly decreased from 118 ± 9 to 106 ± 12 bpm (11%, Fig. 3A) and from $28.0\% \pm 3.6\%$ to $21.4\% \pm 3.5\%$ (24%, Fig. 3B), respectively. These observations show that cardiac imaging is feasible in adult zebrafish and is able to detect ventricular dilatation and dysfunction.

Discussion

Zebrafish as a model to study human cardiac pathology is gaining interest. Although the structural changes after cardiac insult have already been well described in the adult zebrafish,^{7,8,10} the assessment of ventricular function remains a challenge due to the small size of the heart (around 1 mm in diameter). As such, the establishment of noninvasive techniques to study heart function has been highly sought after in the past few years. Indeed, several studies have used high frequency echocardiography, often combined with doppler echography, to assess mechanical and hemodynamic heart function in adult zebrafish.^{11–15}

In this study, a microultrasound catheter designed for intravascular imaging of human subjects has been used to image the zebrafish heart. The microprobe of the IVUS system is easy to manipulate and can be placed on top of the visually beating

FIG. 1. Noninvasive miniaturized cardiac imaging to assess ventricular parameters. (A) Top view of the position of the microcatheter on the ventral part of the zebrafish. (B) An example of B-mode ultrasound image showing the ventricle and the measurement of its diameter. (C) Representative view of the M-mode and determination of ESD and EDD. EDD, end-diastolic diameter; ESD, end-systolic diameter.

thoracic area with ease. Consequently, a rapid localization of the beating ventricle on the B-mode is possible, and can be recognized due to a bright signal intensity in the ventricular wall. The assay can be completed in under 1 min per animal and all tested animals survived the analysis. Having said this, the atrium and the bulbus arteriosus were not clearly defined on the B-mode. This represents a limitation of this device in comparison with previous publications, where all parts of the hearts were observed.^{10–12,14} We suspect that our microprobe is too narrow to allow a full coverage of the whole heart.

Despite this limitation, changes in ventricular size as well as determination of fractional shortening and heart rate during heart failure development are possible with this system. As the zebrafish myocardium is highly trabeculated, a clearly defined ventricular cavity is not visible. Thus, as previously described, the epicardial edge was used to measure heart size in systole

and diastole and to calculate fractional shortening.¹⁶ The significant ventricle enlargement detected by ultrasound imaging after 5 weeks of PHZ treatment was confirmed by the measurement of ventricular surface on excised hearts.

The ventricular enlargement was coupled to a significant decrease in heart rate as previously described in another model of anemia-induced hypertrophy (tr265/tr265 zebrafish mutant).⁴ However, the average heart rate of 118 bpm observed in our study was lower than in other publications (160 bpm at 28°C).¹⁴ This difference may have several explanations. First, tricaine, at a concentration of 160 mg/L, may be a cardiodepressant.¹⁷ Second, the echocardiography in our study was performed at room temperature, which may also be a cardiodepressant. Indeed, it has been shown that a decrease of 10° in temperature can lead to a decrease of 50% in heart rate of zebrafish.¹⁴

Overall, despite the aforementioned limitations, the use of a microprobe for intracoronary imaging in humans can easily be used to evaluate ventricle size and function in adult zebrafish and could be an alternative to other more expensive echocardiography devices.^{11,14,18}

Although echocardiography has been used to assess the heart of zebrafish, we are the first to report the use of coronary ultrasound probes, designed for humans, in the adult zebrafish. The major advantage of echocardiography probes designed for small animals (usually mice) is image quality. The major disadvantages are availability and costs. The use of coronary ultrasound is easy to learn. Therefore, this may facilitate cardiac imaging in the large community of researcher using zebrafish.

In conclusion, miniaturized catheters are well suited for rapid and convenient imaging of the hypertrabeculated ventricle of zebrafish, especially in longitudinal studies. Thus, a novel apparatus for noninvasive imaging to assess the adult zebrafish heart is now available.

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Disclosure Statement

No competing financial interests exist.

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