A Powerful Approach of Live Digital Data Communication in Biomedical Research and Teaching:

Detection of Various Impaired Vascular Perfusion in Zebrafish Knockdown Models by Video Imaging

Ping-Pin Zheng[1], Rob Willemsen[2], Johan M Kros[1]

Departments of Pathology,[1] CBG-Clinical Genetics,[2] Erasmus Medical Center, Rotterdam, the Netherlands

Corresponding: Dr. Ping-Pin Zheng, p.zheng.1@erasmusmc.nl

ABSTRACT

There are various zebrafish models with cardiovascular defects which adequately mimic the impaired circulation and tissue perfusion of various human cardiovascular diseases. Zebrafish embryos/larvae are optically transparent, and the systemic blood circulation can be recorded by using a microscope with video imaging. We detected a series of circulatory defects in our caldesmon and glucose transport 1 knockdown zebrafish models, including arteriovenous (AV) shunting, collateral circulation, AV fusion, vessel bifurcation, reduced or depleted regional perfusion, sinus venous (SV) rupture, and more. The quick detection by simple video imaging of various pathological states of the blood circulation in the living zebrafish embryos/larvae is non-invasive and cost-effective. The method is suitable for large scale screening of altered blood circulation in various zebrafish models with impaired cardiovascular development. This is a powerful approach of live digital data communication in biomedical research and teaching.

Key words: video imaging, blood circulation, zebrafish

1. INTRODUCTION

Zebrafish represents a useful vertebrate model for the study of developmental processes and gene expression, and modeling human diseases. Because the laboratory embryos or larvae are very small and also optically transparent, they are very suitable for whole mount morphological studies including measuring blood circulation in vivo. In the various zebrafish models with abnormal cardiovascular development, defective vascular perfusion and defective circulation, a variety of pathological states of the circulation can be observed, including arteriovenous (AV) shunting, collateral circulation, AV fusion, vessel bifurcation, reduced or depleted regional perfusion, sinus venous (SV) rupture, and more. In this paper we will show a series of circulatory abnormalities which we were able to record in some zebrafish knockdown models with structural defects of vascular development [1,2].

2. MATERIAL AND METHODS

2.1. Animals

All zebrafish protocols were approved by the Institute of Animal Care as before [1,2]. In this study, we used embryos/larvae at developing time points of 1.5 to 3 days post-fertilization (dpf) and controls The knockdown fishes included caldesmon morphants (CaD-MO)[2] and a glucose transport 1 morphants (Glut1-MO)[1].

2.2. Video imaging

Living animals were kept in a petri dish with fresh zebrafish water. No treatment with anesthetics was applied prior to imaging. An inverted microscope coupled with Living Image Software was used for displaying and locating the embryos/larvae. Once defects were traced, we manually used

a regular video camera to record the blood circulation in the spot of interest.

3. RESULTS AND DISCUSSION

The determination and correct interconnection between the arteries and veins as well as the integrity of the specialised vessels are of critical importance for the proper function of the vascular system of vertebrates. Circulatory shunting (CS) is an important sign of functional abnormalities of vascular development. The formation of CS is usually indicative of disrupted arteriovenous (AV) specifications and abnormal vessel bifurcations. Caldesmon (CaD) is evolutionally conserved among vertebrates[3]. The zebrafish homologue is similar to mammalian low-molecular-weight caldesmon (*l*-CaD). The knockdown of *l*-CaD causes serious structural defects in vasculogenesis and angiogenesis in CaD-MO[2]. The blood circulation as a functional parameter was measured by video imaging, which is concomitantly impaired as reflected by formation of collateral circulation (Video 2), AV shunting (Video 3), vessel bifurcation (Video 4) and AV fusion (Video 5) in contrast to the normal control (Video 1). The findings suggest that *l*-CaD is required for proper AV specification, segregation and morphogenesis, which is crucial for normal vascular development and functionality.

In vertebrates, the blood-brain barrier (BBB) is a critical entity for maintaining the brain homeostasis and protects the brain from toxic substances. Disruption of the integrity of the BBB is caused by various pathological processes and has important clinical implications. The differentiated BBB consists of a complex system of barriers represented by highly specialized endothelial cells (ECs) sealed by junctional complexes (adherens junctions [AJs] and tight junctions [TJs]) [1]. Glucose transporter 1 (Glut1), the vertebrate glucose transporter [4], is highly concentrated in tissue barriers consisting of endothelium and epithelium, including the BBB and the blood-retinal barrier (BRB)[5]. BBB and BRB are structurally and functionally similar. In our Glut1MO, both cerebral and ocular circulation is significantly impaired, as shown by highly reduced cerebral perfusion with slow passing of blood cells (Video 7) as compared to the normal control (Video 6), and the ocular circulation shows defective similar to that of brain (Video 9) in contrast to the normal control (Video 8).

Fish have a closed-loop circulatory system. The heart pumps the blood in a single loop throughout the body. In most fish, the heart consists of four parts, including two chambers as well as an entrance and exit. The first part is the sinus venous (SV), a thin-walled sac that collects blood from the fish's veins before allowing it to flow to the second part, the atrium, then the third part (ventricle), and finally the exit (bulbus arteriosus) connecting to the aorta. SV receives blood from the vitelline vein, umbilical vein and common cardinal vein. In our zebrafish Glut1 knockdown model, the structural integrity of SV is dynamically perturbed, manifested as an initial rupture of SV (Video 11) followed by a progressive bleeding (Video 12) while the SV in the control remains intact (Video 10).

In conclusion, simple video imaging allows a quick detection of various pathophysiological phenomena of the blood circulation in living zebrafish embryos/larvae and is non-invasive and cost-effective. It is suitable for large scale screening for altered blood circulation in various zebrafish models with impaired vascular development. This is a powerful approach of live digital data communication in biomedical research and teaching.

Legends of the Video Clips:

Video 1: In the control larva, the blood streams from the dorsal aorta (DA) and the posterior cardinal vein (PCV) are clearly distinct.

Video 2: In contrast to Video 1, in the CaD-MO, collateral circulation is formed involving the DA, intersegmental vessel (ISV), dorsal longitudinal anastomotic vessel (DLAV), ISV back into the DA and further into the PCV.

Video 3: In contrast to Video 1, at multiple levels shunting from the DA into the PCV is noticed.

Video 4: In contrast to Video 1, duplication of the blood flows through parts of the PCV is shown (square) while single linear patterns of PCV have disappeared.

Video 5: In contrast to Video 1, the blood flow from part of the PCV is duplicated (circle). One flow joins into the DA (AV fusion) and another one continues into PCV.

Video 6: Normal circulation with full coverage of the cerebral perfusion in a control (3dpf) (Ventral view, anterior to the right).

Video 7: In contrast to Video 6, reduced coverage of the cerebral circulation with slow passing through of blood cells in a corresponding Glut1MOR (3dpf) (Ventral view, anterior to the left upside).

Video 8: Robust blood circulation in the eye is seen in a 1.5dpf control larva. There is adequate circulation both at the surface and in the intraocular vessels of the eye.

Video 9: In contrast to Video 8, in a corresponding Glut1MOR, the circulation both at the surface and in the

intraocular vessels is arrested irrespective of the presence of normal heart beating, indicating that the arrested circulation is largely induced by a defect of the structural ocular vascular structures rather than defective systemic circulation.

Video 10: Blood flows through a closed SV in a 2.5dpf control. Ventral at the right and anterior to the top.

Video 11: In contrast to Video 10, SV rupture is initiated in a 2.5dpf Glut1 morphant. Ventral at the right and anterior to the left.

Video 12: In contrast to Video 11, SV rupture is being progressive with massive bleeding. Ventral at the top and anterior to the left.

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