Quantitative 4D cardiac imaging for simultaneous determination of calcium dynamics and cardiac function on a beating heart of larval zebrafish in vivo

Yu-Cheng Chuang, Praveen Kumar, Ian Liau*
Institute of Molecular Science and Department of Applied Chemistry
National Chiao Tung University
1001 University Road, Hsinchu 300, Taiwan
Email: ianliau@mail.nctu.edu.tw

KEY WORDS: cardiac function, atrioventricular (AV) conduction, GCaMP

Despite its critical importance, simultaneous determination of the calcium dynamics and the cardiac function of a heart in vivo has never been demonstrated. The difficulty is originated mainly because of the challenge to quantify the local calcium level without artifacts from the moving and contracting cells in a heart and to determine the volumetric change of a rapidly beating heart. Here we demonstrate simultaneous determination of calcium conduction and cardiac function on a beating heart of larval zebrafish in vivo. Transgenic zebrafish larvae (4 days post fertilization) that co-express a calcium sensitive fluorescent protein (GCaMP) and a calcium in-sensitive fluorescent protein (DsRed) were employed, and dual-channel pseudodynamic 3D imaging was employed to construct two sets of 4D images based on the fluorescence of GCaMP and DsRed, respectively. The local calcium transient of within a region of interest (ROI) was represented with the temporal change of the GCaMP signal normalized with that of DsRed within the same ROI whereas essential parameters (such as stroke volume, cardiac output, et al.) of cardiac function were evaluated from the time-varying volume of the cardiac chamber. For demonstration, we study the cardiotoxicity induced by doxorubicin, a potent chemotherapeutic agent. After a treatment of 48 h, the calcium atrioventricular (AV) conduction of larval zebrafish elongated from 120 ms to 170 ms and the cardiac output decreased from 27 nL/min to 7 nL/min. Our approach is distinct from preceding studies in which the calcium dynamic was determined on a heart of zebrafish larvae whose heartbeat was stopped with morpholino [1], and hence no information of the cardiac function was obtained and impairment of the calcium dynamics due to hindered cardiac development may be a concern. In conclusion, our novel approach enables simultaneous determination of the calcium dynamics and cardiac function of a beating heart of larval zebrafish for the first time. The same approach is expected to become a powerful tool for study of cardiac physiology and for assessment of chemicals of cardiotoxicity.

[1] M. Weber, N. Scherf, A. M. Meyer, D. Panáková, P. Kohl, J. Huisken, "Cell-accurate optical mapping across the entire developing heart." *eLife,* **6**, e28307 (2017).