Ultrasound in Med. & Biol., Vol. ■, No. ■, pp. 1–11, 2016
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0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2015.11.026

● *Original Contribution*

TIME COURSE OF ISOFLURANE-INDUCED VASODILATION: A DOPPLER ULTRASOUND STUDY OF THE LEFT CORONARY ARTERY IN MICE

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(*Received* 9 *June* 2015; *revised* 19 *November* 2015; *in final form* 30 *November* 2015)

Abstract—Isoflurane is widely used as vasodilator in studies of coronary flow reserve (CFR) in small animals, but the protocols have not been standardized. This study assessed the time course of the increase in isoflurane-induced flow in the mouse coronary artery by pulsed-wave Doppler measurements at 1% isoflurane concentration maintained for 6 min and then increased to 2.5% for 30 min. Velocity–time integral and velocity peak values were best fitted by the sigmoid model, which allowed derivation of the mean time (*Tt*90 = 14 min) of high-isoflurane needed to reach 90% of the hyperemic plateau value. In subsequent experiments, CFR was measured at 4 min (mean time of literature data) and 14 min of hyperemic response. The 4-min CFR was significantly lower than the 14 -min CFR, and the Bland–Altman plot revealed significant bias of the 4-min CFR against the 14-min CFR. This result suggests that measurements of flow velocity at times shorter than 14 min may be inappropriate for expressing the effective value of CFR. (E-mail: kusmic@ifc.cnr.it) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Coronary flow reserve, Flow velocity, Isoflurane, Vasodilation, Doppler ultrasound, Mice.**

INTRODUCTION

Flow velocity dynamics and flow reserve in coronary arteries are important functional parameters, which condition cardiac function, myocardial viability and ventricular remodeling.

In clinical practice, a reduction in coronary flow reserve (CFR) is often used as a predictor of coronary artery disease (Cortigiani et al. 2007). Alterations of CFR have been also reported in patients with hypertension, dilated cardiomyopathy, diabetes mellitus and metabolic syndrome (Caliskan et al. 2014; Erdogan et al. 2013; Lima et al. 2013; Pirat et al. 2008). CFR is defined as the ratio of the maximal flow velocity induced by a metabolic or pharmacologic stimulus to the resting myocardial blood flow velocity. Several methods have been established for the clinical assessment of CFR: intracoronary Doppler flow, transesophageal Doppler echocardiography, positron emission tomography (PET) and transthoracic coronary Doppler flow (Gan et al. 2013; Naya et al. 2014). However, most of these methods are scarcely relevant in experimental studies, especially on small animal models, because they are invasive or extremely expensive and difficult to reproduce on a very small scale.

Recently, a non-invasive method was developed to measure coronary flow velocity in mice using Doppler ultrasound (Gan et al. 2004; Hartley et al. 2008; Wikstrom et al. 2005). This method is limited by the difficulty in measuring the coronary diameter in small animals and, thus, in obtaining the absolute flow compared with flow velocity. On the other hand, both the current technical challenges and the inappropriateness of using large invasive procedures in longitudinal studies make the use of transthoracic Doppler ultrasound a satisfactory compromise for measurement of CFR in mice. Moreover, Wikstrom et al. (2008), in a comparative study of velocity and volumetric CFR measurements, concluded that velocity-based CFR is the most reliable and robust method and should be used to assess coronary artery function in mice *in vivo*.

Coronary flow velocity can be used to assess CFR in mice during hyperemic coronary responses induced by intravenous adenosine infusion (Saraste et al. 2006; Wikstrom et al. 2005), thus bridging the gap between

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mouse and human for translational studies. However, this procedure is quite troublesome in mice, because the veins are more difficult to cannulate and the tolerated doses and injected volumes are very small (Hartley et al. 2009).

An alternative method used to induce coronary vasodilation is the inhalation of high concentrated isoflurane, one of the most widely used volatile anesthetics (Crystal 1996; Gamperl et al. 2002; Reiz et al. 1983). The use of both adenosine and isoflurane has been proven successful for assessment of the role of coronary microcirculation dysfunction in the development of heart diseases (Saraste et al. 2006; Scherrer-Crosbie and Thibault 2008; Wikstrom et al. 2008). In fact, a close match in results was obtained by direct comparison of the two procedures (Katz et al. 2011; You et al. 2012). These recent studies have prompted investigators to use isoflurane as the preferred coronary vasodilator because of its convenience by obviating tail vein cannulation, thus making this procedure truly non-invasive.

Moreover, because isoflurane is the anesthetic usually used during echocardiographic imaging sessions in mice, the combined use of adenosine infusion and the fluorinated anesthetic during coronary flow measurements may affect the correct assessment of CFR (Larach and Schuler 1991). In addition, albeit the procedure of assessing the resting coronary flow velocity at 1% isoflurane concentration and the hyperemic response at 2.5% (Hartley et al. 2007, 2008, 2009, 2010) is generally accepted, a standardized protocol to assess CFR in mice, as well as a detailed time course of the vasodilation of the coronary artery under higher isoflurane concentration, is lacking. Indeed, in previous studies, the time between the shift to 2.5% isoflurane and the measurement of maximal flow velocity was very variable (3–10 min) or not specified at all (Hartley et al. 2007, 2011; You et al. 2012).

The work described here, using high-frequency ultrasound, was aimed at studying the time course of left coronary artery (LCA) vasodilation induced by shifting from 1% to 2.5% isoflurane concentration and assessing the best-fitting model of the data, thus deriving crucial information on the optimal time of analysis at which to assess the maximal vasodilation to appropriately calculate the CFR. Finally, we calculated and compared the CFR appraisals obtained at two different points in the time course of the hyperemic response: at the time predicted by the model and at an average time as described in the literature.

METHODS

Animals

Adult male C57BL/6 (n = 20, mean age: 6 mo; n = 6, mean age 15 mo) were obtained by in-house breeding (progenitors were purchased from Harlan Italia, Udine, Italy).

Animals were housed on a 12-h light/dark cycle and allowed free access to food and water. The study was performed in accordance with European Directive 2010/63/UE and Italian law (D.L 26/2014). The study was approved by the ethics committee of the Experimental Biomedicine Center, National Research Council, Pisa, Italy.

Left coronary flow velocity measurement

Mice were initially anesthetized with 2% isoflurane (IsoFlo, Abbott Laboratories, Maidenhead, UK) in 100% oxygen using an induction chamber connected to a scavenger canister. After induction, mice were placed on a temperature-controlled board and kept on 1.5% isoflurane during investigation. The limbs were coated with conductive paste (Signa Cream, Parker Laboratories, Fairfield, CT, USA) and taped on the electrocardiograph electrodes to monitor heart rate and respiration frequency. Also, body temperature was monitored and maintained between 36°C and 37°C. The chest was shaved using hair-removing cream (Nair, Church & Dwight Canada, Mississauga, ON, Canada), to minimize ultrasound attenuation, and coated with acoustic coupling gel (SonoSite Cogel, Comedical Sas, Trento, Italy). Flow velocity in the left coronary artery (LCA) was measured from a modified parasternal long-axis view using a 40-MHz probe (MS550, VisualSonics, Toronto, ON, Canada) and recorded pulsed-wave Doppler image sequences (VEVO2100, VisualSonics), with the angle correction value kept between 0° and 20° (depending on the ultrasound projection) and the sample volume placed in the midproximal segment of LCA.

Time course of hyperemic response induced by isoflurane concentration

Ten 6-mo-old mice were used to study the time course of left coronary vasodilation induced by increasing the concentration of isoflurane. After visualization of the LCA in the image window, isoflurane concentration was set and maintained at 1% for 6 min (resting condition); isoflurane concentration was then switched to 2.5% (hyperemic condition) and maintained over 30 min.

Pulsed-wave Doppler images were acquired every 2 min and elaborated using the software provided with the ultrasound equipment (VEVOLab, VisualSonics). The envelope of the diastolic phase of the velocity signal was manually outlined, and both the velocity–time integral (VTI) and velocity peak (VP) were assessed in five cardiac cycles, chosen during consecutive expiration phases. The final VTI and VP values were calculated for each time point as the average of these five measurements.

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