Original paper

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Coronary sinus flow measured by pulsed Doppler ultrasound is a powerful indicator of coronary blood supply – a pig heart in vitro study.

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Abstract

Aims: To evaluate the correlation between the coronary sinus flow and the infusion volume in the coronaries and assess the performance of coronary sinus flow in predicting coronary artery occlusion in an isolated pig heart. **Material and methods:** The coronary sinus flow was measured in 16 isolated pig hearts by pulsed Doppler ultrasound. The correlation between the coronary sinus flow and the infusion volume in different coronary artery was analyzed, and the performance of coronary sinus flow in predicting different coronary artery occlusion was deducted. **Results:** There were no statistically significant differences between the coronary sinus flow and the infusion volume in different coronary artery ($p>0.05$). The correlations between the coronary sinus flow and the infusion volume in left anterior descending coronary artery (LAD), left circumflex coronary artery (LCX), LAD and LCX, and LAD, LCX and right coronary artery (RCA) were all higher than 0.85 ($p<0.01$), and those of RCA, LAD and RCA, and LCX and RCA were between 0.6 and 0.8 ($p<0.05$). The areas under the receiver operating characteristics curve (AUC) were all higher than 0.90 ($p<0.05$) in predicting any two coronaries occlusion (<50% and 100%) and three coronaries occlusion (<50%) with a >85% sensitivity and specificity. Excepting RCA mild occlusion (<50%), AUCs in predicting one coronary occlusion (<50% and 100%) were between 0.7 and 0.9, with >80% sensitivity and specificity. **Conclusions:** The coronary sinus flow measured by pulsed Doppler ultrasound can effectively and exactly reflect the infusion volume in coronaries, which is a powerful indicator of coronary blood supply.

Keywords: coronary sinus flow, coronary artery infusion, Doppler ultrasound; isolated heart, pig

Introduction

The coronary sinus is the major venous collection system for the coronary arteries [1], a superficial vascular structure located in the sulcus between the left atrium and the left ventricle. It extends from the greater cardiac vein valve to its ostium in the right atrium and receives most of the ventricular epicardial veins [2]. Although a large number of clinical and experimental studies have validated the efficiency of retrograde coronary sinus perfusion [3-9], the studies on the diagnostic performance of coronary sinus flow detected by Doppler echocardiography in coronary artery disease are not yet rich enough. Over the last decade, the coronary flow reserve in the coronary sinus has been used to diagnose severe coronary artery stenosis by transesophageal Doppler echocardiography due to inadequate visualization of the coronary arteries [10-12]. Recently, our studies showed that coronary sinus flow depicted by transthoracic Doppler echocardiography at rest can detect coronary artery stenosis in non-hypertensive [13], hypertensive [14], and diabetic hypertensive patients. These studies including transesophageal and transthoracic Doppler echocardiography still just depicted the coronary sinus flow (reserve) in the case of angiographically proven normal or stenotic coronary artery, but the exact relationship between coronary perfusion and coronary sinus flow remains unknown.

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Animal models are used frequently to investigate the diagnosis and therapy techniques of heart disease, and the pig has similar heart anatomic structure and hemodynamic performance to human [15,16]. Therefore, in this study, we performed a pig heart in vitro study to evaluate the precise correlation between the coronary sinus flow and the infusion volume in the coronaries and to assess the performance of coronary sinus flow in predicting coronary artery occlusion in order to further confirm the results of our in vivo studies and provide a useful foundation for future studies.

Materials and methods

Preparation of the isolated hearts

Our study was approved by the local human research ethics committee of The First People's Hospital of Yancheng (No.2014019). Sixteen adult angiographically proven healthy China Landrace pigs (Changba Farm, yancheng, China; 8 males and 8 females; mean age: 1.5±0.3 years, range: 1.2-1.8 years; mean weigh: 96.8±12.3kg, range: 85-112 kg) were involved in this study. After jugular vein injection of anesthesia with 95% ethanol (2ml/kg), fresh hearts (mean weigh: 407.6±56.3g, range: 352-467kg) were quickly removed from the thoracic incision along the left rib cage. Heparin (0.2 IU/g) was administered. The proximal ends of the coronaries (left anterior descending coronary artery, LAD; left circumflex coronary artery, LCX; and right coronary artery, RCA) were punctured by the disposable infusion needles (Specifications: 0.7, Suyun Medical Equipment Co., Ltd., Lianyugang, China), and the needles were connected with an artificial triple tee which was made of three together linked tees (Discofix 3SC, Escholzmatt, Switzerland) (fig 1).

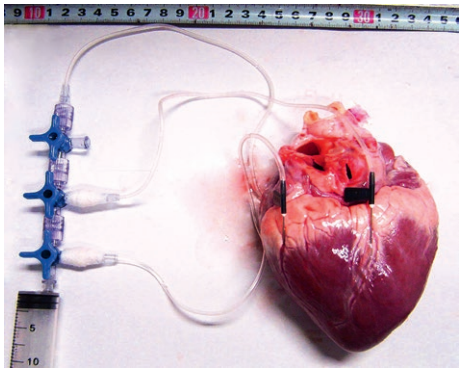


Fig 1. Preparation of the isolated hearts. The proximal ends of the coronaries (LAD, LCX, and RCA) were punctured by the disposable infusion needles, and the needles were connected with an artificial triple tee which was made of three together linked tees.

Coronary infusion

When LAD was infused, LCX and RCA were occluded; when LCX was infused, LAD and RCA were occluded, and so on. For one coronary infusion study, LAD, LCX and RCA were injected with 1-2ml sterile and pyrogen-free saline solution (Huayu, Pharmaceutical Co.,Ltd. Wuxi, China). For more coronaries infusion study, the combination of LAD and LCX, LAD and RCA, LCX and RCA, and LAD and LCX and RCA were injected with 3-5ml sterile and pyrogen-free saline. Coronary artery complete occlusion (100%) was fulfilled by closing the tee switches, and coronary artery mild occlusion (<50%) was completed by lightly ligaturing the proximal coronary artery. Coronary artery complete occlusion (100%) and mild occlusion (<50%) was confirmed by two-dimensional and color Doppler ultrasonography.

Doppler ultrasound measurements and analysis of coronary sinus flow

A complete two-dimensional ultrasonography and Doppler study was performed in all subjects using a commercially available ultrasonic system (Vivid E9; General Electric Medical Systems, Milwaukee, WI, USA) equipped with a 9L harmonic 5-10 MHz variable frequency phased array transducer. The isolated pig hearts were placed in the left front and right rear position so that the great cardiac vein and coronary sinus can easily display. The transducer was placed on the migration of great cardiac vein and coronary sinus along the sulcus between the left atrium and the left ventricle. In the course of coronary perfusion, sterile and pyrogen-free saline was injected in a constant speed. The transducer was manipulated to visualize the mouth of the coronary sinus. The coronary sinus diameter was measured at a 0.5 cm distance from the mouth (fig 2a). A pulsed-wave sample

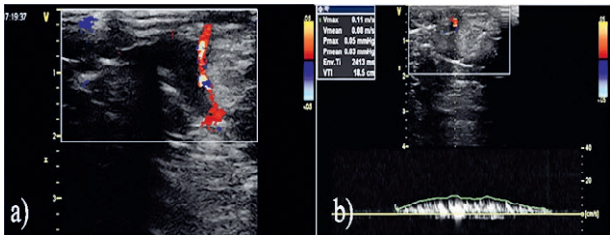


Fig 2. Coronary sinus shown from the migration of great cardiac vein and coronary sinus along the sulcus between the left atrium and the left ventricle using color Doppler ultrasound and the measurement of the coronary sinus diameter (A) the flow spectra shown in coronary sinus caused by the coronary infusion using pulsed Doppler ultrasound and the measurement of Doppler parameters for the coronary sinus flow by digitized Doppler spectral envelopes (B). Vmax, peak velocity; Vmean, mean velocity; Pmax, peak pressure gradient; Pmean, mean pressure gradient; Env.TI, duration of the measured envelope; and VTI, velocity time integral.

volume (2 mm) was placed at a 0.5 cm distance from the mouth, and then rotated by a small amount (Doppler angle between the ultrasound beam and vessels <20°) to obtain the optimum Doppler flow signals, and spectral recordings of the flow were made. Finally, the antegrade phase of coronary sinus flow moving into the right atrium was analysed. The velocity time integral (VTI, cm) were determined through digitized Doppler spectral envelops (fig 2b). The coronary sinus flow was calculated according to the formula: flow $=\pi\times D^2/4\times VTI$, where π is the ratio of the circumference of a circle to its diameter, and D is the diameter of the coronary sinus [10]. The average value of three spectral and planimetric envelopes was used.

Reproducibility

Intraobserver variability was assessed in 16 subjects by repeating the measurements on two occasions (3 days apart) under the same basal conditions. To test the interobserver variability, the measurements were performed on the same subject by a second observer blinded of the first observer's results.

Statistical Analysis

Data were expressed as the mean±SD. The differences between the two groups were tested using an unpaired two-tailed t test. A receiver operating characteristic curve analysis was used to evaluate and compare the performance of the coronary sinus flow in predicting a significant coronary artery occlusion and its validity parameters (sensitivity, specificity and Youden index). Agreement between intraobservers and interobservers in the measurement of coronary sinus diameter and VTI were assessed and compared by Bland-Altman Plots. A value of $P<0.05$ was considered statistically significant. All statistical analysis was performed with SPSS version 16.0 software for Windows (SPSS Inc, Chicago, IL).

Results

Visualization of the coronary sinus

Adequate gray-scale visualization and a good spectral Doppler flow of the coronary sinus as well as their technically adequate measurements for analysis were obtained in all the subjects.

Coronary sinus flow and injection volume in coronaries

As shown in Table I, there were no statistically significant differences between the coronary sinus flow and the infusion volume in different coronary artery ($p>0.05$). No flow was measured by Doppler ultrasound when three coronaries (LAD, LCX and RCA) were completely blocked.

Table I. Coronary sinus flow measured by pulsed Doppler ultrasound and injection volume in different coronary artery.

Coronary artery	Coronary sinus flow (ml)	Injection volume (ml)	p-value
LAD	1.21±0.78	1.11±0.22	0.62
LCX	1.01±0.63	1.38±0.52	0.22
RCA	1.81±0.54	1.85±0.37	0.89
LAD+LCX	2.15±1.17	2.50±0.61	0.46
LAD+RCA	2.81±1.01	2.92±0.85	0.41
LCX+RCA	2.78±1.07	3.29±0.76	0.33
LAD+LCX+RCA	3.80±1.24	4.36±0.85	0.35

LAD – left anterior descending coronary artery; LCX – left circumflex coronary artery; RCA – right coronary artery

Table II. Correlations between coronary sinus flow measured by pulsed Doppler ultrasound and injection volume in different coronary artery.

Coronary artery	Correlation coefficient	p-value
LAD	0.93	<0.001
LCX	0.96	<0.001
RCA	0.62	0.013
LAD+LCX	0.89	0.003
LAD+RCA	0.76	0.007
LCX+RCA	0.67	0.011
LAD+LCX+RCA	0.85	0.005

LAD – left anterior descending coronary artery; LCX – left circumflex coronary artery; RCA – right coronary artery

Correlation between the coronary sinus flow and the infusion volume in coronaries

As shown in Table II, the correlations between the coronary sinus flow and the infusion volume in LAD, LCX, LAD, and LCX (LAD+LCX), and LAD, LCX, and RCA (LAD+LCX+RCA) were all greater than 0.85 ($p<0.01$), and those of RCA, LAD and RCA (LAD+RCA), and LCX and RCA (LCX+RCA) were between 0.6 and 0.8 ($p<0.05$).

Discriminant analysis of coronary sinus flow for predicting a significant coronary artery occlusion

The results of discriminant analysis of coronary sinus flow for predicting a significant coronary artery occlusion were shown in Table III. The areas under the receiver operating characteristics curve (AUCs) were all greater than 0.90 ($p<0.05$) for respective best cut off value in any two coronaries (LAD+LCX, or LAD+RCA, or LCX+RCA) occlusion (<50% or 100%) and three coronaries (LAD+LCX+RCA) occlusion (<50%), with >85% sensitivity >85% specificity and a >0.75 Youden index. Excepting RCA mild occlusion (<50%), AUCs in predicting one coronary (LAD, or LCX, or RCA) occlusion (<50% or 100%) were between 0.7 and 0.9 for respective best cut off value, with >80% sensitivity and specificity and >0.63 Youden index.

Table III. Area under the receiver operating characteristics curve, optimal cut-off points, and validity parameters of different coronary sinus flow in predicting coronary artery occlusion in different coronary artery occlusion

Variables	AUC (95% CI)	Cut off value (ml)	Sensitivity (%)	Specificity (%)	Youden index
RC RCA mild occlusion (<50%)	0.73(0.58-0.88)	3.92	76.9	71.5	0.484
RCA complete occlusion (100%)	0.88(0.69-1.06)	3.36	87.5	85.7	0.732
LAD mild occlusion (<50%)	0.74(0.47-1.00)	3.45	83.6	80.9	0.645
LAD complete occlusion (100%)	0.78(0.50-1.06)	3.32	85.7	85.7	0.714
LCX mild occlusion (<50%)	0.72(0.47-0.98)	3.58	82.4	81.3	0.637
LCX complete occlusion (100%)	0.80(0.53-1.06)	3.45	86.4	85.2	0.716
LCX and RCA mild occlusion CA complete occlusion ty and a (<50%)	0.95(0.91-0.98)	2.13	85.8	89.2	0.75
LCX and RCA complete occlusion (100%)	0.98(0.94-1.03)	1.99	88.9	100	0.90
LAD and RCA mild occlusion (<50%)	0.99(0.97-1.01)	1.96	89.4	88.6	0.763
LAD and RCA complete occlusion (100%)	0.995(0.99-1.00)	1.87	97.6	93.8	0.914
LAD and LCX mild occlusion (<50%)	0.92(0.75-1.08)	1.69	89.5	86.8	0.76
LAD and LCX complete occlusion (100%)	0.98(0.79-1.17)	1.58	92.3	89.8	0.82
LAD, LCX, and RCA mild cclusion (<50%)	0.97(0.77-1.16)	2.04	90.2	88.7	0.79

LAD – left anterior descending coronary artery; LCX – left circumflex coronary artery; RCA – right coronary artery; AUC – area under the curve; ROC – receiver operating characteristics; CI – confidence interval

Inter/intra observer variability

Intra observer and inter observer agreement in the measurement of coronary sinus diameter was fairly good: intraclass correlation coefficient (ICC) was 0.94 (0.83–0.97), 0.95 (0.85–0.99) for inter and intra observer, respectively. Inter and intra observer agreement in the measurement of VTI was still good: ICC was 0.92 (0.77–0.96), 0.93 (0.75–0.97) for inter and intra observer, respectively.

Discussions

This is the first report on the precise correlation between the coronary sinus flow measured by pulsed Doppler ultrasonography and the coronary infusion in an isolated heart model. The results of our study indicate that the coronary sinus flow measured by pulsed Doppler ultrasound is able to directly and exactly reflect the coronary perfusion, and a reduced coronary sinus flow is a powerful predictor of coronary artery stenoses.

Up to date, only a few of articles about the relationship on the coronary sinus flow reserve or coronary flow reserve between coronary angiography results in the literature. They are all in vivo studies using transesophageal and transthoracic Doppler echocardiography [10-14,17,18], and none of these studies provided the precise correlation between the coronary sinus flow and the coronary infusion. Compared with in vivo studies, our study had some possible advantages. First, we used isolated hearts, which were significantly different from the living hearts. Isolated hearts avoided the influences of the heart rate, heart rhythm, atrial, ventricular and pulmonary artery pressure, mitral and tricuspid valvular regurgitation. Second, we used fresh hearts (in 2 hours after the hearts were isolated from the living body) and heparin was simultaneously administered, which ensured an unobstructed coronary circulation. Third, we used artificial triple tees to replace the coronary artery ligation, their exactly controllable stopcocks ensuring a complete occlusion situation. As for mild occlusion, adjunctive coronary artery ligation was only employed. This design simplified the operational procedures. In this model, we can evaluate the coronary sinus flow in a situation of each coronary artery infusion and any of their combination. Four, the coronary sinus flow measurement was very easy due to the lack of any interference from heart movements and the possibility to examine using a nearly 0° Doppler angle between the ultrasound beam and vessels. This is also a great advantage compared to the in vivo studies.

In our study, there was a significantly higher correlation between the coronary sinus flow measured by pulsed Doppler ultrasound and the coronary artery infusion in the left coronary artery comparing to the right coronary artery. This finding is related to the heart anatomic structure, the coronary sinus being the major venous collection system for the coronary arteries, especially for the left coronary arteries. Although the coronary sinus flow in the single coronary artery (except RCA) more closely correlated with the coronary artery infusion than that of two or three coronary arteries, the coronary sinus flow in the group of two or three coronary artery occlusion has a more powerful performance in predicting coronary artery stenoses than that of single coronary artery occlusion. At the same time, we found that the coronary sinus flow in

the group with complete coronary artery occlusion has more powerful performance in predicting coronary artery stenoses than that of mild coronary artery occlusion. These results indicate that reduced coronary sinus flow is a powerful predictor of coronary artery stenoses, especially for more coronary arteries and severe coronary artery disease, which is agree to our clinical findings.

Our study had some limitations. First, we used isolated hearts, which could not imitate the condition of the living heart. Second, there was interspecies anatomic differences between human and pig; further feasibility studies of the pig heart model are needed. Third, the number of participants was limited. New data need to be collected in subsequent studies. Four, we used sterile and pyrogen-free saline to replace the fresh blood, which caused possible errors due to the different dynamic characteristics between them.

Conclusions

This is the first study on evaluating the precise correlation between the coronary sinus flow measured by pulsed Doppler ultrasound and the coronary infusion in an isolated pig heart model. Our study demonstrates that there are high correlations between the coronary sinus flow and the coronary infusion, and coronary sinus flow has a powerful performance in the diagnosis of coronary artery stenoses. Although some limitations mentioned above, this method still holds considerable clinical promise for the diagnosis, treatment and follow-up of coronary artery disease, which also provides a useful foundation for future studies.

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Conflict of interest: none

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