Hirosaki Med. J. 71 : 101—107, 2021

ORIGINAL ARTICLE

Preliminary study on local medical treatment of aortic valve calcification: Drug delivery to aortic valve in rat

Zaiqiang Yu[1)], Xu Liu[1)], Kazuyuki Daitoku[1)], Ken-Ichi Furukawa[2)], Ikuo Fukuda[1)], Shigeru Motomura[3)], and Kazuhiko Seya[4)]

Abstract

Background

Aortic valve stenosis (AVS) is the most common heart valve disease in elderly society. The effective treatment for AVS is surgical aortic valve replacement but with extremely invasive. Although transcatheter aortic valve replacement also is performed for highly risk patients with minimally invasive, it has to resolve the problem of bioprosthetic valve durability in future. However, there is no effective medical treatment established for AVS to inhibit acceleration of aortic valve calcification.

Objectives

We aimed to confirm whether the ascending aorta is the best location for AVS to innovate local and selective medical therapy or not.

Methods and Results

After anesthetized by isoflurane, the wild type rat was stablished on operating table. Opening chest to heart by median sternotomy approach, the catheter was inserted into left ventricular from apical for body perfusion. We injected saturated solution of amido black 10B (AB) into ascending aorta above sino-tubular junction about 0.5 cm from aortic valves without aortic dissection observed, and body perfusion was performed by saline containing heparin in order to prevent embolism happened in vascular. After 30 minutes, the heart with the ascending aorta was extracted. We used optical microscope to check the existence of AB at around of aortic valves or not. Interestingly, AB was detected at around of aortic valves and valsalva sinus of the aorta and myocardium in all rats.

Conclusions

These results suggested that AB injected is delivered from the ascending aorta to aortic valves through vasa vasorum. These data provide very important evidences for local medical therapy development for AVS patients.

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Key words: aortic valve stenosis; aortic valve calcification; local medical therapy.

Introduction

Aortic valve stenosis (AVS) is the most frequent heart valve disease in the elderly[1, 2], with higher prevalence up to 4.6% with increasing age after over 75 years old in the world, which is accelerated by aortic valve calcification (AVC) deteriorating with aging[3, 4]. Two effective treatments are surgical aortic valve replacement (SAVR) with extremely invasive, and transcatheter aortic valve replacement (TAVR) performed for AVS patients with high risk to open surgery but with the problem of bioprosthetic valve durability. Because AVC becomes progressing in AVS patients without any symptoms in several decades, non-invasive medical drugs treatment for AVS to inhibit acceleration of AVC is required impatiently.

[1)] Department of Thoracic and Cardiovascular Surgery, Hirosaki University Graduate School of Medicine.
[2)] Department of Orthopaedic Surgery, Hirosaki University Graduate School of Medicine.
[3)] Department of Pharmacology, Hirosaki University Graduate School of Medicine.
[4)] Department of Vascular Biology, Hirosaki University Graduate School of Medicine.

Correspondence: Z. Yu, MD, and K. Seya
Received for publication: August 6, 2020
Accepted for publication: October 19, 2020

Although several groups have focused on the mechanism of AVC in AVS[5, 6], there is no effective non-invasive treatment established to inhibit acceleration of AVC. Our research group also focus on mechanism of AVC and development of medical treatment to avoid operative intervention for AVS patients. Recently, we developed some effective substances to prevent AVC in cellular level[7, 8]. However, it is possible that the systemic administration of AVC inhibitors disturbs the bone homeostasis. From these reasons, we need to find out the best administration route to get the most treatment effectiveness for drugs presently or developed in future.

Ascending aorta (AA) is continuous to valsalva sinus of the aorta which connecting with aortic valves and the left ventricle. Because AA is the nearest to aortic valves and easy to access from femoral arteries like TAVR, there is a possibility that the AA is one of available position to perform local medical therapy for AVS patients, but we have to avoid some severe complications like aortic dissection. However, there is no report indicating the drug delivery from AA to aortic valves.

In this study, we investigated whether the drug administered into the wall of AA is delivered to aortic valve and valsalva aorta in rat.

Methods

Materials

Solvent amido black 10B and Sudan black was purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). Male Wistar rats at 20 weeks of age were purchased from Charles River Japan Inc. (Tokyo, Japan). All experiments were performed according to the Animal Experiment Guidelines of the Laboratory of Animal Experiments for Hirosaki University School of Medicine. The animals were maintained at a temperature of 22 ± 2°C and relative humidity of 57% on a 12-h light/12-h dark (lights on 08:00-20:00), and had free access to water and food.

Administration of drug into the wall of the ascending aorta (AA)

After isoflurane anesthetized, male Wistar rat (350~410 g) was put on operating table (n = 4). Opening chest approach to heart by median sternotomy, the injected catheter was inserted into left ventricular from apical for body perfusion using infusion pump and heparinized saline (50 IU/mL) under 15 ml/hr. Saturated amido black 10B (AB) saturated with saline (approximately 5 μL) was directly injected into the wall of AA media above sino-tubular junction away from aortic valves about 0.5 cm (Fig 1). After 30 min of total perfusion, the heart with AA was extracted.

Histochemical staining

After the extraction of heart with AA, which was fixed in 10% buffered formalin for 24 hours and then embedded in paraffin. Paraffin-embedded specimens were examined for morphology with hematoxylin-eosin (HE) stain. After HE stain was performed, we used optical microscope to check existence of AB at around of aortic valves and valsalva sinus of the aorta or not.

Results

The distribution of amido black 10B (AB) after injection into the wall of ascending aorta (AA)

To confirm the distribution of AB, we investigated using optical microscope in non-stained sample. Figure 2A showed the normal myocardium without AB injection. Although almost of injected AB localized in aorta (Fig 2B), a part of this agent moved to valsalva sinus via the aortic wall. We confirmed that AB injected delivered to valsalva sinus of the aorta after 30 min of injection. Figure 2C showed the

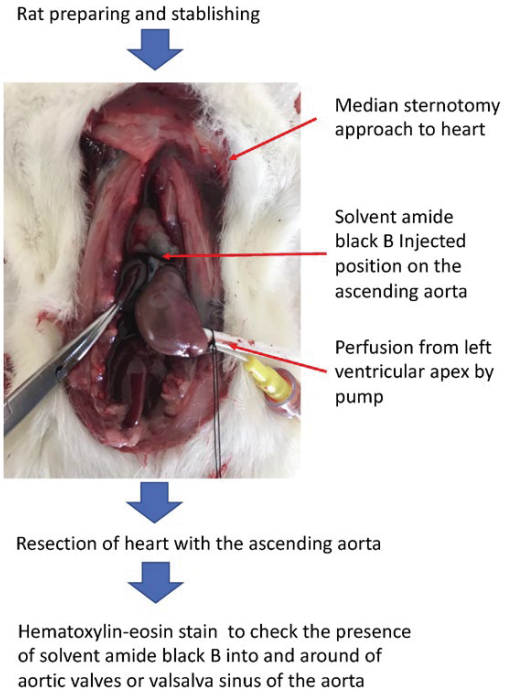

Fig. 1 After isoflurane anesthetized, the wild type rat was stablished on operating table. Opening chest approach to heart by median sternotomy, the injected catheter was inserted into left ventricular from apical for body perfusion using infusion pump and saline under 15 ml/hr. We injected solvent amido black 10B (AB) into the wall of the ascending aorta above sino-tubular junction away from aortic valves about 0.5 cm.

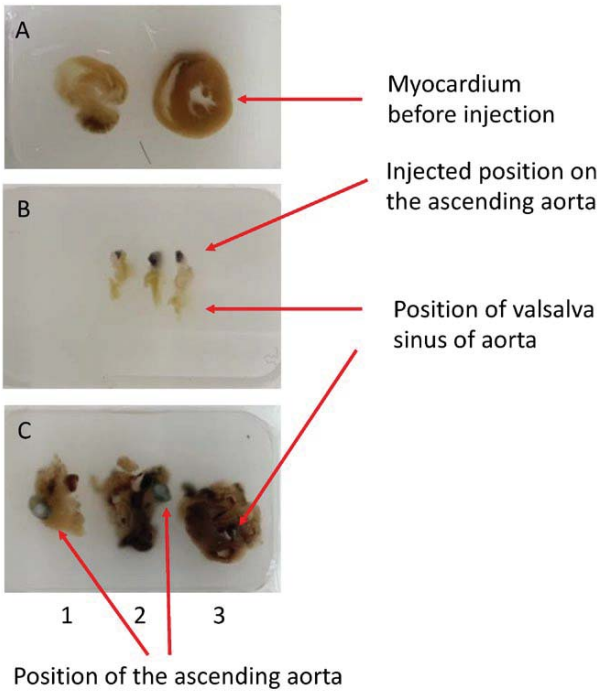

Fig. 2 The ascending aorta and heart were sectioned and placed in paraffin (A, B , C). Injection position (B) and stained area (C) by AB in each region was showed.

stained area by AB (as described in the figure legend) in ventricle (Figure 2C-3) and ascending aorta (Figure 2C-1, -2) after AB injection at the aortic wall. These results indicate that AB injected into the wall of AA delivered to ventricular myocardium and valsalva sinus of the aorta.

AB was found in and around of aortic valve

To establish the effective local medical therapy for AVS, we investigated whether AB injected into AA wall (Fig 3A) distributes into aortic valve or not in detail. Optical microscope was used for confirming the presence of AB in aortic valve. There was no any dissection found at injected position of the ascending aorta and valsalva sinus of the aorta. AB was strongly stained AA wall near by valsalva sinus (Fig 3C) and reached to valsalva sinus of aorta (Fig 3 H). Interestingly, many AB depositions were found at aortic valves (Fig 3C), myocardium (Fig 3E, F, G) and adipose tissue (Fig 3H) in all rats by HE stain (golden arrows). Figure 3B and 3D indicated normal valsalva sinus of aorta with aortic valve and myocardium respectively without AB injected as a negative control. These data suggested that there is drug delivery route from the wall of AA to aortic valves. So we need to administer the drugs to the around of AA for inhibiting AVC acceleration.

Discussion

Aortic valve stenosis (AVS) is the most common heart valve disease in the elderly[1, 2], the most viable treatment is surgical aortic valve replacement (SAVR) with large invasive. Recently, transcatheter aortic valve replacement (TAVR) is performed for elderly AVS patients

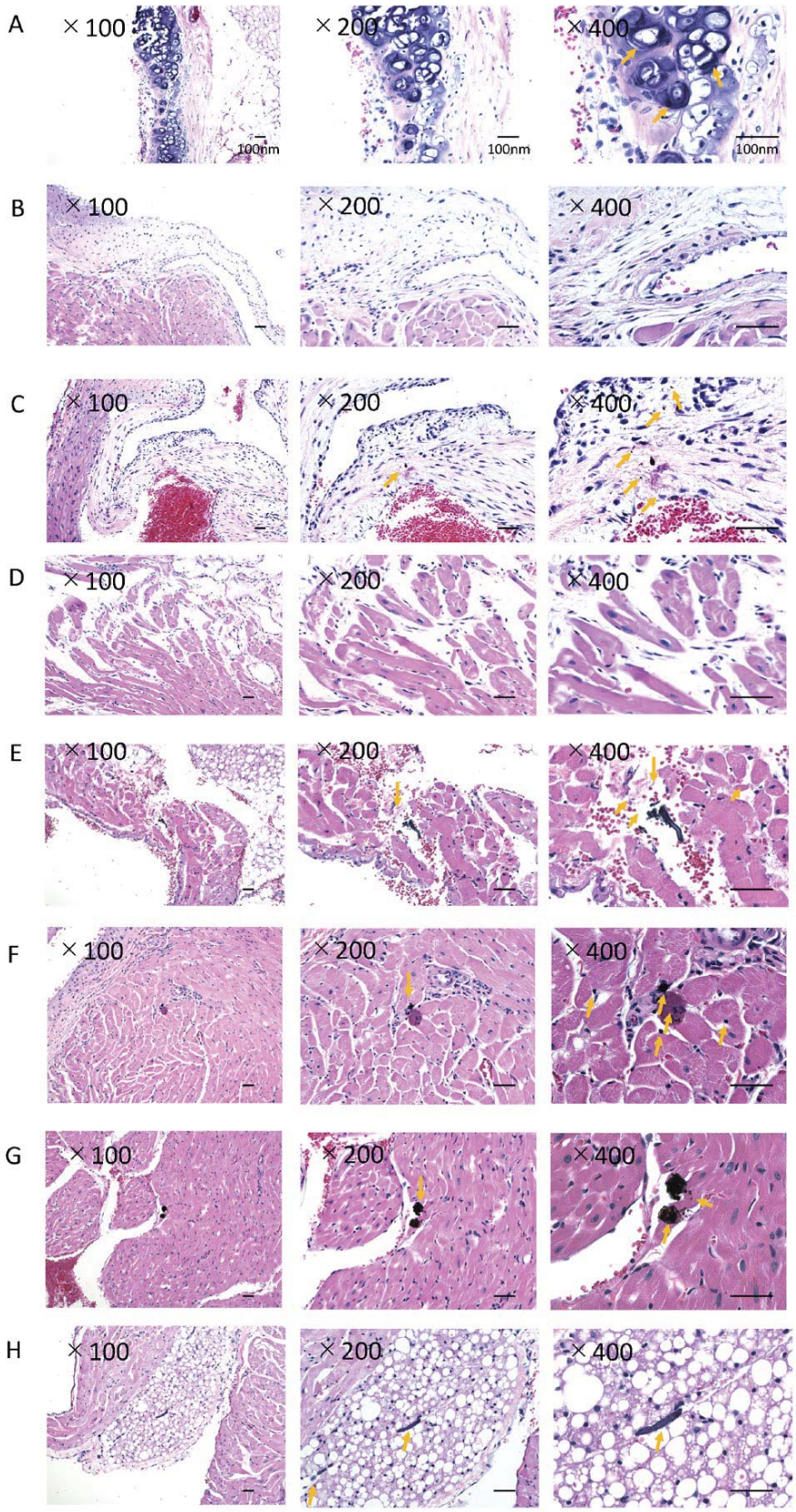

Fig. 3 Hematoxylin-Eosin (HE) stain was performed. No AB injected tissue: valsalva sinus of aorta (B), myocardium (D). AB was found at myocardium or with adipose tissue (E, F, G, H), the ascending aorta (A) and valsava sinus of aorta with aortic valves (C) in all rats (golden arrows).

or patients with high risk to open surgery. However, calcification of prothesis aortic valves is another important issue after TAVR[9]. After SAVR or TAVR, we always use warfarin to prevent prosthesis valves from thrombosis formation for patients combined with atrial fibrillation[10]. Warfarin (WFN) is the most common anticoagulation drug for valvular atrial fibrillation with valve disease, deep vein thrombosis and postoperative anticoagulation for preventing embolism of prosthetic valve, which inhibiting vitamin K-dependence clotting factor. However, several studies had showed that intake WFN long-term induced acceleration of arteries and valves calcification with enhancing risk of bleeding event year by year[11]. Prosthesis valve is implanted for elderly patients, who can avoid intaking WFN long time postoperative, but these prosthesis valve has durability problem damaged by calcification occurred.

On the other hand, for AVS patients, surgical treatment was improved with less invasive like TAVR than open surgery, but there was no effective medical treatment for 34% patients who could not tolerated surgical intervention[12]. According to our recently research, we found various drug candidates which inhibits ectopic calcification of aortic valve[7, 8]. We further demonstrated that there were some things in a part of Kampo products which has effect on inhibiting acceleration of tumor necrosis factor-α/high-phosphate induced valvular calcification (data not shown). However, there is no effective local route of administration to select but not oral or venous injection. The systemic administration of calcification inhibitors via oral or venous injection will induce undesired toxic effects for calcified tissues such as bone, tooth, and so on. Further, we could not inject drugs in aortic valves directly, because aortic valve is very thin and always moving very rapidly.

According to anatomy of aortic valve, which are connecting to left ventricular myocardium and valsalva sinus of the aorta directly, and valsalva sinus connecting to ascending aorta (AA). Although left ventricular myocardium and valsalva sinus are connecting to aortic valves, we think that it is too difficult to access, and too difficult to do some intervention for aortic valves. So, we think that AA near to aortic valve is the best position for the constitutive administration of calcification inhibitors, it is easy to access but with risk of acute aortic dissection which need operation performed. However, it is unclear whether the drugs administration into and around of aorta is delivered to aortic valve.

In this study, we injected amido black 10B (AB) into the wall of ascending aorta without aortic dissection to confirm whether drug transits to aortic valves or not. Interestingly, we found that there were lot of deposited AB at aortic root and left ventricular myocardium. We investigated by using another lipid soluble Sudan black, however, this pigment was also delivered to aortic valve, but insufficient (data not shown). The wall of aorta includes many small nutrient blood vessels[13]. These results indicated a possibility that AB injected in the wall of AA is delivered to the root of aortic valve and myocardium through valsalva aorta. However, it is unknown whether AB in the wall of AA is delivered through nutrient small blood artery. In the next study, we should investigate the movement of substance with fluorescent dye in the wall of aorta using more large animals.

Although we found that drugs can transit to aortic valves from AA, we could not perform drugs injection to ascending aorta directly because it is with high risk of acute aortic dissection, which is the worst complications and need emergency surgery performed to prevent sudden deaths induced by rupturing. However, the device of continuously drug-eluting ring or stent graft placed by catheter at inner of ascending aorta, or we think that drug-eluting sheet

placed on the out of AA is effective solution to select[14], the effect of these solution for admining drugs to aortic valves need to be further investigated.

Conclusions

In conclusion, we demonstrated a possibility that drugs also could deliver from ascending aorta to aortic valves like AB. This investigation is basal level to establish the drug delivery to aortic valve. These results would be effective for developing now medical therapies for aortic valve stenosis.

Acknowledgments

This study was performed as JSPS KAKENHI Grant Numbers 16K10619, 16K10449, 17K10916, 18K16381, and 19K09089.

We would like to thank professor Tadaatsu Imaizumi for revising this paper. Department of Vascular Biology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Study conception: ZY, KS
Data collection: ZY, XL, KS
Analysis: ZY, KS
Investigation: ZY, KS
Writing: ZY, XL, KS
Funding acquisition: None
Critical review and revision: All authors
Final approval of the article: All authors
Accountability for all aspects of the work: All authors

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