ScienceDirect

IFAC PapersOnline CONFERENCE PAPER ARCHIVE

IFAC PapersOnLine 51-27 (2018) 237–240

Model and verification of the NO distribution in curved blood vessel

Huiting Qiao[1,2],Cong Liu[1], Hongjun Zhao[1], David Dagan Feng[3,2]

1 School of Biological Science and Medical Engineering, Beihang University, Beijing, China (e-mail:qht@buaa.edu.cn)
2 Beijing Advanced Innovation Centre for Biomedical Engineering, Beihang University, Beijing, China
3 School of Information Technologies, Sydney University, Sydney, Australia(e-mail: dagan.feng@sydney.edu.au)

Abstract: Curved blood vessels are the high incidence of cardiovascular and cerebrovascular diseases. The asymmetric nitric oxide (NO) distribution in blood vessels may maintain or regulate the curvature of blood vessels. To understand the distribution feature of nitric oxide in curved blood vessels, 3-D models were established to predict the distribution of NO in curved vessels. Animal experiments were also executed *in vitro* to verify the NO distribution. Simulation results show that the distribution of nitric oxide in the curved blood vessel is not uniform. The distribution of NO is different between the inner and outer sides of curved blood vessels. Immunohistochemical results showed that eNOS was mainly expressed in the vascular endothelium, and eNOS at the outside of the bend was significantly more than that at the inner side. This study is expected to open up new translational research to address vascular disease risk assessment and treatment.

Keywords: multi-physics model, nitric oxide, curved blood vessel, eNOS expression

1. INTRODUCTION

Nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) plays a significant role in cardiovascular system (Zhang, White et al. 1994; Plank, Wall et al. 2007; Forstermann and Sessa 2012). NO can regulate vascular tone, inhibit thrombosis, inhibit inflammatory, and regulate smooth muscle proliferation. The role of NO to diastolic smooth muscle tone and to regulate blood flow has gotten more and more attention of scholars and clinicians. Nitroglycerin, nitroprusside can cause vasodilation and change vascular resistance by releasing NO. The vascular tone regulation function of NO has close relation with local NO concentration distribution.

In our former work (Han, Qiao et al. 2014), we have found that the tortuosity proportion of cerebral middle artery among people may change according to the age. We hypothesize that the morphology of blood vessel may change for some reason, and NO maybe one of those reasons. Thus we want to know how NO distributes in curved blood vessels, and if it may give the possible to aggravate the tortuosity. In this paper, we established a 3-D model to simulate the multi-physics phenomenon of the generation, diffusion and consumption of NO. And then we use animal experiments to verify the model.

The concentration detection of NO can be divided into two categories: direct detection technology and indirect detection technology. The direct detection of NO mainly refers to the direct biochemical detection of NO activity, such as using nitrate reductase to detect serum NO concentration. This method is simple and easy to operate, but it just gives an overall assessment of NO concentration. The indirect detection refers to detection of NO concentration by detecting the expression of nitric oxide synthase (NOS). For example, immunohistochemistry (IHC) is used to detect the expression of eNOS in endothelial cells (Kuo, Wang et al. 2004; Javanmard, Nematbakhsh et al. 2009). This method can accurately find the local eNOS distribution. In this study we picked up a vascular ring to discover the local NO concentration distribution in curved vessels.

2. METHOD

2.1 Multi-physics model

3-D models were established according the reasonable morphology of human straight vessel and curved vessel. The 3-D model is composed of three parts: lumen, vascular wall and tissue part (Fig. 1). Vascular wall includes endothelium and smooth muscle cell.

Blood flow in the lumen was assumed to be steady, and steady-state incompressible. Navier-Stokes equations, as in (1), were used to simulate the Hemodynamics in the lumen, where $\mathbf{u}$ is the fluid velocity vector, p is the pressure, μ is viscosity of blood in lumen, 0.0035kg/(m·s)

$$\begin{aligned} \rho(\mathbf{u}\cdot\nabla)\mathbf{u} + \nabla p - \mu\Delta\mathbf{u} = 0 \\ \nabla\cdot\mathbf{u} = 0 \end{aligned} \tag{1}$$

10.1016/j.ifacol.2018.11.636

NO is released by the endothelium, and diffuses into both lumen and the surrounding tissues. In the lumen, mass transport equation including convection and diffusion was used to simulate NO distribution, as in (2). In the vascular wall and tissue, diffusion equation was used, as in (3).

$$D_{\mathrm{NO}}\Delta c_{\mathrm{B}} - \mathbf{u}\cdot\nabla c_{\mathrm{B}} - k_{\mathrm{B}}c_{\mathrm{B}} = 0 \quad (2)$$

$$D_{\mathrm{NO}}\Delta c_i - k_i c_i = 0 \quad (3)$$

where D_{NO} is the diffusion coefficient of NO, $3.3\times10^{-9}\mathrm{m^2s^{-1}}$(Lancaster 1997), c_{B} is the concentration of NO in blood, k_{B} is the reaction rate of NO in blood, $23\mathrm{s^{-1}}$(Liu, Wang et al. 2014). In (3) i=W,T, that means in the vascular wall and in the tissue, the reaction rate of NO in vascular wall equals to that in the tissue, $k_{\mathrm{W}}=k_{\mathrm{T}}=0.01\mathrm{s^{-1}}$.

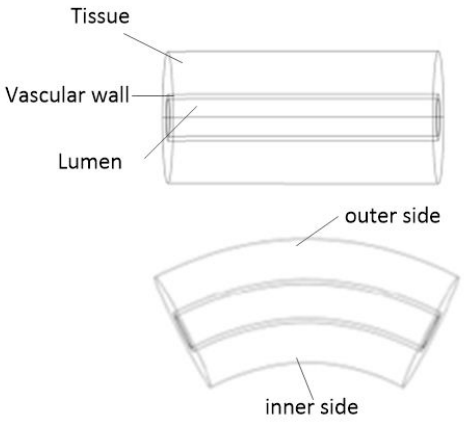

Fig. 1. Schematic of 3-D curved blood vessel models.

The concentration of NO changes continuously in nearly all the region except the boundary of vascular wall towards blood side (W-B boundary), where lies endothelium. Endothelium is the place where NO generated.

$$\boldsymbol{N}_{\mathrm{B}}\cdot\mathbf{n}_{\mathrm{B}} - \boldsymbol{N}_{\mathrm{W}}\cdot\mathbf{n}_{\mathrm{B}} = R_{\mathrm{NO}}h \quad (4)$$

Where N_{B} is the flux of NO from blood side at B-W boundary, N_{W} is flux of NO from vascular wall side at B-W boundary, $\mathbf{n}_{\mathrm{B}}$ is normal vector at B-W boundary, h is the thick of endothelium, 2μm. The production rate of NO, R_{NO} is regulated by wall shear stress (WSS). According to the measurement of Andrew(Andrews, Jaron et al. 2010), there is a hyperbolic relation between the production rate of NO and WSS.

$$R_{\mathrm{NO}} = R_{\mathrm{NO,basal}} + R_{\max}\frac{|\boldsymbol{\tau}_{\mathrm{w}}|}{|\boldsymbol{\tau}_{\mathrm{w}}|+a} \quad (5)$$

Where $R_{\mathrm{NO,basal}}$ is the basal production rate of NO, $R_{\max}$ is the maxium production rate of NO, $\boldsymbol{\tau}_{\mathrm{w}}$ is WSS calculated from hemodynamic model, and α is a constant.

The vascular wall was assumed to be a no-slip rigid wall. The calculations were performed using an advanced commercial finite element software platform COMSOL 5.1 (COMSOL, Inc.). Multi-physics simulation modules, including laminar flow and transport of diluted species, were used.

2.2 Animal experiment

Wistar SPF clean grade rats (male, 300±10g, 8 weeks) were ordered from Beijing Vital River Laboratory Animal Technology Co. The scheme of the animal experiment has been shown (Fig.2). All procedures were reviewed and approved by School of Biological Science and Medical Engineering's Animal Care and Use Committee.

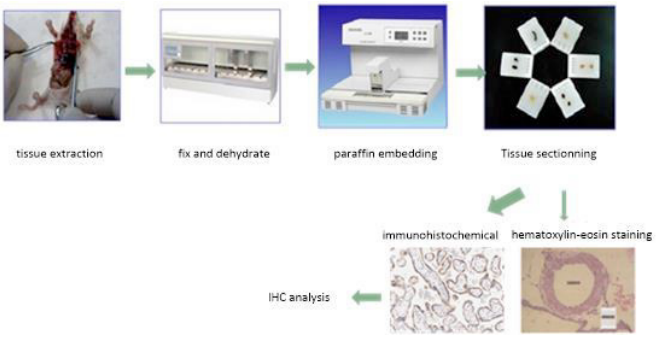

Fig. 2. Scheme of the animal experiment.

First, three fasted rats were sacrificed. After the anticoagulant is added, a segment of aorta, including the typical curved vessel without efferent arteries, was quickly removed and rinsed with PBS buffer. Another straight vessel segment was picked up from cerebral artery as control group. Then the rinsed vessel segments were placed in 4% PFA Fix solution to fix. Twenty-four hours later, the vessel segment was carefully removed using tweezers. For curved vessel, a gap mark was made on the inner side of the curved vessels by scissors to distinguish the bending side of curved vessels. Using anhydrous ethanol dehydration dehydrates vessels for 10 hours. And then the vessels were paraffin embedded and made into tissue sections. After paraffin section dewaxing to water, antigen retrieval, blockading of endogenous peroxidase, blocking of BSA, plus primary antibody, secondary antibody, DAB colorization and dehydration and sealing, as conventional procedure was followed, and the images were collected and analysed by microscopic examination. The morphology of blood vessels was observed by HE staining. The results of immunohistochemistry (IHC) were stained blue with hematoxylin , and the expression of eNOS was brown.

3. RESULTS

2.1 Nonuniform distribution of NO in curved vessels

The numerical simulation results show the concentration distribution of NO in each model. The concentration of NO is not uniform in lumen, vascular wall and tissues. The highest NO concentration appears at the boundary between vascular wall and blood flow lumen, where the endothelium lies. From

the vascular wall to the lumen, the NO concentration decreases very quickly. At the same time the NO concentration also decreases from the vascular wall to tissue, but not as quickly as in lumen side. These results consist with the published NO distribution(Chen, Buerk et al. 2007).

When only the transection of the vascular wall is considered, difference can be seen between straight vessel and curved vessel. The distribution of NO in straight vessel is axial symmetric, while that in curved vessel is not (Fig. 3). The NO concentration at the inner side is lower than that at the outer side.

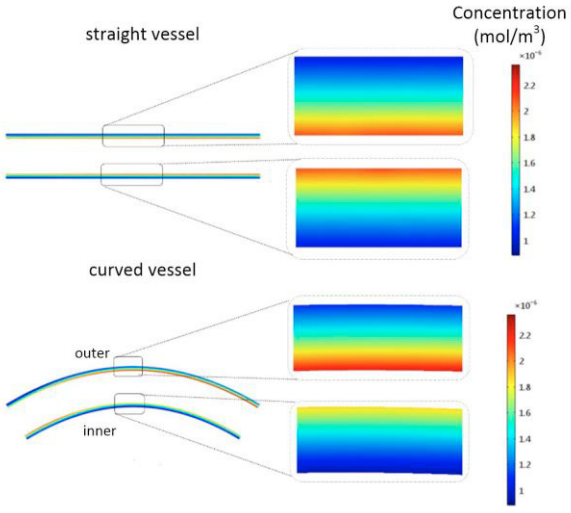

Fig. 3. Distribution of NO in straight vascular wall and in curved vascular wall.

2.2 Nonuniform expression of eNOS in curved vessels

To verify the results of numerical simulation, HE stain and IHC were executed on both straight and curved vessels. For straight blood vessel, eNOS is expressed at the boundary between vascular wall and blood flow lumen where endothelium lay. The eNOS expressed at the endothelium of straight vessel almost uniformly (Fig. 4).

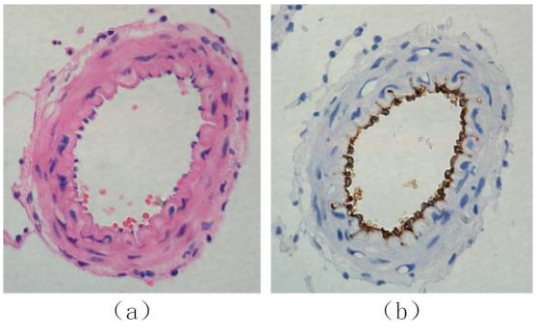

Fig. 4. HE stain (a) and IHC (b) results of the straight vessel.

For curved vessels, the gap mark is clear to show which side is the inner side. eNOS is also expressed at some part of the boundary between vascular wall and lumen part. The expression of eNOS in curved vessel transection is not symmetric. The expression of eNOS at outer side is more obviously than that at the inner side (Fig.5).

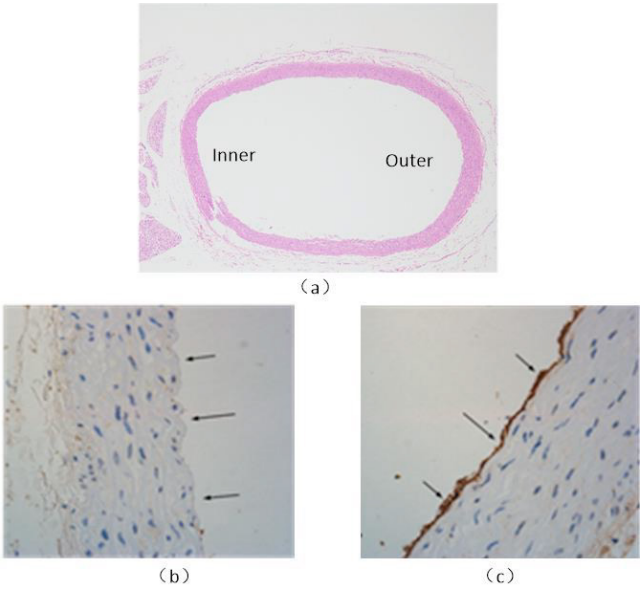

Fig. 5. HE stain (a) and IHC results (b) for inner side, (c) for outer side of the curved vessel.

To make a semi-quantitative compare, the value of integrated optical density (IOD) was used. Image-Pro Plus 6.0 software is used to select the same brown as the standard for judging the expression of eNOS. Each result of IHC is analysed to obtain the integrated optical density value of inner side and outer side separately. From the results of IOD for 3 subjects (Table.1), the IOD at outer side is obviously higher than that at inner side.

Table 1. IOD compare

subjects	1	2	3
Inner	189.53	154.13	639.75
outer	1936.16	1423.04	3456.46

4. DISCUSSION

The curved blood vessels are always the high incidence of cardiovascular and cerebrovascular disease, just as atherosclerosis. Asymmetric NO distribution in vascular wall maybe one of the factors to maintain or exacerbate the tortuosity of blood vessel. This paper focused on the concentration distribution of NO in curved vessels. On one hand, multi-physics numerical model was used to simulate the distribution feature of NO in vascular wall, lumen and tissue. On the other hand, *in vitro* animal experiment was executed to verify the results of numerical simulation. Both results show that the NO concentration at inner side is lower than that at outer side in curved blood vessel.

Since NO is easily consumed by reaction in body environment, the real time detection of multiple points is very difficult. There are many factors to affect the distribution of NO, for example flow field and mass transport control the NO concentration distribution at the same time. Thus numerical multi-physics modelling is an efficient method to study NO distribution in human body. The verification of numerical simulation is necessary, so this study take animal experiment into account.

The difference of NO concentration between inner side and outer side could be caused partly by the difference of WSS. For the character of flow field in curved vessels, the WSS at the inner endothelium is less than that at the outer endothelium. Other factors just as convection may also have influence. Further study should be done to find the factors effecting the tortuosity of blood vessel.

5. CONCLUSIONS

By numerical simulation and animal experiment, the paper found NO concentration distribution is not uniform in curved blood vessels. NO concentration at inner side is much lower than that at outer side. The difference of NO concentration may maintain or even exacerbate the tortuosity of blood vessel.

ACKNOWLEDGEMENT

This work was supported in part by the 111 project (B13003), National Key Research and Development Program (2016YFC1304305) and National Science and Technology Support Program (2015BA102B00).

REFERENCES

Andrews, A. M., D. Jaron, et al. (2010). "Direct, real-time measurement of shear stress-induced nitric oxide produced from endothelial cells in vitro." Nitric Oxide **23**(4): 335-342.

Chen, X., D. G. Buerk, et al. (2007). "A Model of NO/O2 Transport in Capillary-perfused Tissue Containing an Arteriole and Venule Pair." Annals of Biomedical Engineering **35**(4): 517-529.

Forstermann, U. and W. C. Sessa (2012). "Nitric oxide synthases: regulation and function." Eur Heart J **33**(7): 829-837, 837a-837d.

Han, J., H. Qiao, et al. (2014). "The three-dimensional shape analysis of the M1 segment of the middle cerebral artery using MRA at 3T." Neuroradiology **56**(11): 995-1005.

Javanmard, S. H., M. Nematbakhsh, et al. (2009). "l-Arginine supplementation enhances eNOS expression in experimental model of hypercholesterolemic rabbits aorta." Pathophysiology **16**(1): 9-13.

Kuo, Y. R., F. S. Wang, et al. (2004). "Nitrosoglutathione improves blood perfusion and flap survival by suppressing iNOS but protecting eNOS expression in the flap vessels after ischemia/reperfusion injury." Surgery **135**(4): 437-446.

Lancaster, J. R., Jr. (1997). "A tutorial on the diffusibility and reactivity of free nitric oxide." Nitric Oxide **1**(1): 18-30.

Liu, X., Z. Wang, et al. (2014). "Nitric oxide transport in normal human thoracic aorta: effects of hemodynamics and nitric oxide scavengers." PLoS One **9**(11): e112395.

Plank, M. J., D. J. Wall, et al. (2007). "The role of endothelial calcium and nitric oxide in the localisation of atherosclerosis." Math Biosci **207**(1): 26-39.

Zhang, F., J. G. White, et al. (1994). "Nitric oxide donors increase blood flow and reduce brain damage in focal ischemia: evidence that nitric oxide is beneficial in the early stages of cerebral ischemia." J Cereb Blood Flow Metab **14**(2): 217-226.