Studying Flow Mediated Responses in Isolated Vasculature:
Webinar Q&A Report

Q: What specific cerebral vessel is Dr. Thorin using for his studies?

DR. THORIN: From the mouse, we use two posterior cerebral arteries and two middle cerebral arteries. This yields four segments per mouse. We do not see differences in reactivity to flow or acetylcholine between the two different arteries.

Q: Did I hear correctly during the explanation of the system setup that the MOPS solution is not oxygenated? Why not?

DR. HERRERA: I don't recall MOPS solution being discussed in this presentation. That being said, a MOPS-buffered saline does not require oxygenation. This is because you would set the pH of the buffer by titration using, for example, NaOH. The main purpose of oxygenating, or more specifically "bubbling", a bicarbonate buffered physiological saline solution is for pH control. The gas mixture in this case must contain 5% CO2. As Dr. Thorin pointed out, some labs bubble bicarbonate-buffered saline solutions with 5% O2 in 95% O2. However, it is preferable to bubble with 5% CO2 with 21% O2, balance of N2. This is so that the oxygen tension in the saline solution is normoxic, instead of hyperoxic. 95% O2 solutions result in a hyperoxic oxygen tension, which may effect redox pathways.

Q: Do you use a superfusate or perfusate to evaluate compound effects (e.g. phenylephrine, acetylcholine, etc.)?

DR. HERRERA: Reagents such as phenylephrine and acetylcholine are typically applied to the bath superfusate, rather than infused through the vessel lumen.

DR. THORIN: You will need to perfuse large molecular weight compounds if you need to assess an intraluminal effect only, such as the effect of an antibody on an endothelial response.

Q: How long can a vessel tolerate (be viable) flow conditions within an experimental protocol?

DR THORIN: In arterioles typically used in the arteriography, we only test the effects of flow up to one hour. I remember studies showing that myogenic tone could be maintained up to 24 hours. Flow-induced arteriolar reactivity, however, may be more damaging to the endothelium and we typically never go further than one hour of experiment when investigating flow or the cumulative effects of an agonist on arteriolar reactivity. Thus, we only perform one protocol on one arteriolar segment.

Q: Is it possible to study two different vessels in the same set up at the same time?

DR. HERRERA: Living Systems Instrumentation does make a dual vessel chamber, which we refer to as our model CH-2. In the CH-2 vessel chamber, you can mount two vessels simultaneously. You can run two experiments at the same time, provided you have all necessary equipment (e.g. two pressure servo controllers, two flow control pumps, two perfusion pressure monitors, etc.). The thing to remember, however, is that you can only record arterial diameter from one vessel at a time. This is because the vessel chamber sits atop an inverted microscope, which only has a single objective lens. So to obtain readings of arterial diameter from two preparations, one needs to move the vessel chamber back and forth on the microscope stage at the appropriate times during the experiment to obtain diameter recordings from both vessels. The alternative approach is to run one experiment to completion, while a second vessel is equilibrating, and then move to dual vessel bath to the second vessel position once the first experiment is completed.

Q: Is there a shear stress range you like to stay within during the typical experiment?

DR THORIN: The physiological shear stress level is estimated to be around 15 Dynes/cm^2. Therefore, I would suggest that below 5 and above 30 Dynes/cm^2 the values are not relevant. For reference, please refer to the following papers:

Langille BL and O'Donnell F, 1986. Science 231, 405–407

La Barbera M, 1990. Science 249, 992-1000

Malek AM, et al, 1999. JAMA 282, 2035–2042

Bolduc et al, 2013. Am J Physiol 305, H620–H633

Q: What is an acceptable level of resistance between the cannulae during a flow experiment (delta P)? Say you have matched cannulae within 1 µm each, how can you further reduce resistance in the system?

DR. HERRERA: Typically, you should see no more than 3-5 mmHg pressure differential between the proximal and distal pressure transducers when the cannula are appropriately matched. Reduce unnecessary tubing as much as possible. Also, use tubing with an inner diameter larger than the glass pipettes to minimize resistance artifacts.

Q: I use Mesenteric arteries - when I use a flow rate of 10-20 µL/min the endothelium gets damaged, however, anything below that does not start the flow system using the pressure myography technique of 1 chamber. Any suggestions?

DR. HERRERA: It sounds like you may be dealing with an equipment issue. If you are using a pump to deliver your intraluminal flow, you may need to decrease the size of the tubing installed in the pump to optimize performance of the pump. Using a smaller size tubing in the pump will allow you to effectively deliver lower infusion rates.

Q: If recording raw data from the transducer 1 (so it is not averaged between 1 and 2), is it possible to only have one transducer? What are the implications of only using one transducer of the vessel if only using pressure control (no flow)?

DR. HERRERA: This approach is not ideal if you are using an automatic system such as the one offered by Living Systems and discussed in the webinar. You may have problems that result in a pressure drop across the vessel, and you would not be able to determine this if you are only using one transducer. The Pressure Servo Controller should be operating to maintain intravascular pressure at a constant level. In the presence of flow, you will have a pressure drop across the vessel, as the vessel and glass pipettes act as a flow resistor. If you are only sensing pressure on one side of the vessel, intravascular pressure will actually be higher or lower than your pressure set point, depending on what side of the vessel you are measuring pressure. If, however, you have a static preparation with no intraluminal flow, you only need one pressure transducer. The pressure on either side of the vessel can be measured in this case. There is no pressure drop across the vessel, as there is no flow. A pressure drop across the vessel will only occur in the presence of intraluminal flow.

Q: In an automatic setting in which the vessel is pressurized, how does the PSS solution flow through the system when intraluminal flow is applied?

DR. HERRERA: The Living Systems Instrumentation automatic system uses a miniature peristaltic pump to deliver the PSS solution through the system.

Q: The endothelium is very hard to remove in human arteries. We have tried air, flow, soap, pulling hair through, hair with a knot, triton x and are now trying collagenase. Do you have any ideas/experience with other ways?

DR. THORIN: The only method we use is either air bubbles for 30 seconds, or inserting a human hair inside the lumen before mounting the artery on the arteriography, but the latter is not easy. Usually with the bubbles it works. If the artery is large enough, you could try inserting a wood peg and rubbing it.

Q: So can we actually simultaneously control pressure and flow rate with a pressurized arteriography system?

DR. HERRERA: Yes, you can simultaneously control both pressure and flow. Specialized equipment is necessary to do so. Attention must be paid to controlling the intraluminal pressure. In the Mechanic system that Dr. Thorin described, the intravascular pressure must be measured using the servo-null technique for any given pair of glass micropipettes at a given size arterial segment. With an Automatic system such as the one from Living Systems Instrumentation, intravascular pressure is constantly estimated by taking the average of the perfusion pressure on the proximal and distal side of the vessel.

Q: What is the cost of the automatic system?

DR. HERRERA: Price of a fully equipped automatic system can vary, depending on what accessories you already have in the lab, how you choose to measure arterial diameter, how you choose to control bath temperature and pH, and so on. We can follow up with you specifically to learn more about your specific needs.

Q: Can you study effects of shear stress in a wire myograph preparation?

DR. THORIN: Yes, technically you can. In the late 80's, John Bevan's group in Vermont tested this. A pipette was approached very near one open side of the segment and flow was initiated. Dilations were obtained on pre-constricted vessels. However, because the wire myograph records isometric tensions, no change in shape (diameter) was induced by flow: thus, shear stress was not normalized. These experimental conditions are closer to those obtained with cultured cells on which a shear stress is imposed.

Q: What kinds of responses will you see if you increase shear stress in a vessel in which the endothelium has been damaged? How do you know if the endothelium has been damaged, or if that is just the normal response of the vessel?

DR. THORIN: First, you could always test the function of the endothelium at the beginning of your protocol by inducing dilation with a single dose of acetylcholine on a pre-constructed segment with phenylephrine. The first part of your question is more difficult: normally, in the absence of endothelium or with a dysfunctional endothelium, flow should not induce dilation or this dilation would be reduced. But, this will hold true if the level of tone is average and if the level of shear stress is "physiological" that is between 5 and 25 Dynes/cm^2. At high levels of tone, shear stress may induce a contraction. At high levels of shear stress, a contraction may be obtained as well. You should get the reference values for your type of artery, practice, make sure you are "reproducible" and you should get your answer with reasonable confidence.

Q: Does shear stress affect responses to vasoactive compounds like endothelin?

DR. THORIN: Yes it will. Shear stress should stimulate NO production, a powerful dilator of ET-1-preconstricted arteries. If you pre-constrict the arterial segment with a depolarizing solution containing high K^+, shear stress is likely to be less efficient at increasing arteriole diameter.

Q: Is it normal to see flow induced dilations, or flow induced constrictions?

DR. THORIN: At a physiological shear stress value and an average vascular tone, you should get dilation. Shear stress-induced constriction may occur at high tone and/or at high flow rates (high shear stress values). Alteration of the contribution of the various endothelium-derived relaxing factors using selective antagonists or in vessels isolated from knockout mice may change the normal responses.

Q: Does flow induced dilation change according to the contractile agent used?

DR. THORIN: Different agonists will lead to different levels of shear-stress-dependent response. Shear stress will usually promote NO release that is a powerful dilator on various agonist but not all such as a depolarizing solution of thromboxane A2, at least in our laboratory.

Q: Does flow induced dilation stimulate conducted vasodilation mechanism in small resistance arteries?

DR. THORIN: This is difficult to answer because fluid flows from a high to a low resistance point but simultaneously on the whole arterial territory involved. There is no need for such a mechanism unlike for what can be seen with the local release of a vasoactive factor that can induce retrograde conducted dilations. In vivo, however, such a local dilation will generate a local drop in pressure and therefore an increase in flow will be generate and dilate the whole vascular territory.

Q: Have you done work on larger conducting vessels and do you stretch the vessels to "in-vivo" length prior to reactivity studies?

DR. THORIN: Yes, with the arteriography with have tested renal arteries and carotid arteries of the mouse. We usually stretch them 10% which is likely less than in vivo for the carotid artery but about right for the renal artery.

Q: How does a vessel respond to oscillatory shear stresses?

DR. THORIN: We do not know! There is no system that can reproduce ex vivo these conditions. Based on clinical data and some older studies, the use of a non-pulsatile extra-corporal circulation during coronary artery by-pass surgery is associated with more inflammation than with a pulsatile pump. A better renal perfusion has also been reported in pulsatile compared to constant flow pump. Some reference can be extracted from our latest publication attempting to study this specific question. (Raignault A, Bolduc V, Lesage F, Thorin E. Pulse pressure-dependent cerebrovascular eNOS regulation in mice. J Cereb Blood Flow Metab. 2016 Jan 28. pii: 0271678X16629155)

Q: You mentioned that shear stress is constant in the body, but what about pulsatile changes in flow?

DR. THORIN: The average shear stress is constant in the body. During pulse pressure and flow, the diameter of the artery will passively increase and thus shear stress, that is inversely proportional to the r^3, will tend to remain constant.

Q: Is it a problem if the flow direction is not the same as it would be physiologically? (What happens during reverse flow?)

DR. THORIN: I do not know. To my knowledge, this has never been formally tested. However, we try to maintain the original flow direction when we mount the arteries in the arteriography.

Q: Couldn't increasing shear stress be causing the release of ROS and superoxide which might be preventing the dilation?

DR. THORIN: Maybe at high shear stress but in our recent study we observed that flow was rather "anti-oxidant" compared to agonist-induced endothelium-dependent dilation in mouse cerebral arteries. (Raignault A, Bolduc V, Lesage F, Thorin E. Pulse pressure-dependent cerebrovascular eNOS regulation in mice. J Cereb Blood Flow Metab. 2016 Jan 28. pii: 0271678X16629155)

Q: If there is a branch/hole in the vessel that is not obvious, how does that affect shear stress when using the pressure servo-control system?

DR. HERRERA: You should try to isolate side branches when you mount the vessel. You should always do a leak test, in which you close off the vessel at a constant pressure and see if the vessel maintains a constant pressure in the absence of any external perturbations. If so, it is a good indication that there are no leaks in the vessel. If the intravascular pressure continuously drops, it is an indication that there is a side branch, which will interfere with the ability to accurately control pressure and flow. Sometimes, it is possible to correct a leaky vessel by moving the leaky portion above your vessel ties. In many cases, this is not possible, and the leaky vessel should be discarded.

Q: Would you expect vascular tissue (e.g. coronary arteries) to have differential responses to vasoactive compound/drugs that cause drug induced vascular injury vs. vasoactive compounds/drugs that do not?

DR. THORIN: I would guess so. Using a drug that induces vascular damage, flow-mediated vascular responses will be altered.

If you have additional questions for Dr. Gerry Herrera or Dr. Éric Thorin regarding content from their webinar or wish to receive additional information about solutions for in-vitro isolated blood vessel research please contact them by phone or email:

Catamount Research
& Development Inc.,
Living Systems Instrumentation

+1 802-863-5547
gerry.herrera@catamountresearch.com

Professor,
Department of Surgery
Université de Montréal

+1 514-376-3330 ext. 3589
eric.thorin@umontreal.ca

LIVING SYSTEMS INSTRUMENTATION
innovations for vascular research

insidescientific

Copyright 2016 InsideScientific and Living Systems Instrumentation. All Rights Reserved.