Webinar Q&A Report:
Experimental Considerations when Planning Chronic Models of Cardiovascular Disease in Rodents

1. **How precise is implant technology for hypertensive studies?**

   Dr. Tim Hacker: In terms of accuracy and repeatability, implants are far and away better than any non-invasive method. We have found that non-invasive blood pressure measurements, like tail-cuff methods, can produce inconsistent data even though the animals are awake and not under anesthesia.

   When you compare acute invasive measurements with implantable telemetry, we tend to see much smaller differences between groups because of anesthetic effects. For measuring blood pressure in small animals, I think telemetry really is the gold standard.

2. **How should I filter relevant data from the data measured by telemetric device?**

   Dr. Tim Hacker: I tend to look at the full file first and look for events. If nothing immediately pops out to me, I stratify it by night and day. If time of day differences are not apparent, I use software to find the periods of the highest heart rates and the lowest heart rates. We usually find the most interesting events at those points. If all that data matches what we see looking at ECG or different interval times, then we just pick one to present. If they don't match then you might want to present those data separately. Depending on your model, transition times, such as just before of just after mice go to sleep or wake up, could also be important. If you use automated analysis, you can also set different heart rate limits or pressure limits to help make sense of your data.

3. **What is the impact of animal gender on cardiovascular research?**

   Dr. Tim Hacker: There is a clear gender difference in both mice and rats. We have done quite a bit of research on this topic and we think it's an estrogen factor. In our research, ovariectomized females skew more towards what males look like. In pulmonary hypertensive studies with right heart failure, estrogen seems to have an even more protective effect.

A challenge with our research now is that, with the NIH saying researchers need to use both genders, sample size needs to go up. We have seen studies with 5 males and 5 females, and there does not seem to be much of a difference between the treated and untreated groups. It might be that the females are pushing the averages between groups closer together, so you don't see a significant difference. So if you do use both males and females, make sure you have a high enough sample size to sort out whether there is a difference between males and females.

We see a little less of a gender difference with myocardial infarction models, but certainly with some pressure overload models, there does seem to be differences between males and females.

4. **In chronic mouse pulmonary hypertension models (i.e. hypoxic PH model or other models), how can one achieve right ventricular failure in mice?**

Dr. Tim Hacker: We have had the most the most success with pulmonary arterial banding. Surgically, it is technically difficult, but we very consistently get right heart failure with pulmonary arterial banding.

The Sugen-Hypoxia model is another good model. The trick there is you have to push the length of hypoxia to a much longer length and induce fairly severe hypoxia, somewhere around 8% and 2-3 weeks in the hypoxic chamber to get right heart failure.

5. **How do you know when you have achieved heart failure, and how do you achieve it in the right or left ventricle?**

Dr. Tim Hacker: Rather than assume heart failure if there is hypertrophy, I go with a more stringent model of heart failure, requiring a deficit in function and changes in systolic or diastolic function. We use echo to look at structural changes: we want to see dilation or at least a change in the ratio of wall thickness to the chamber dimension to see some kind of hypertrophy levels, then we will look for a reduction in ejection fraction.

If we're looking at pressure we should see a drop in blood pressure that is unsustainable. Higher heart rates combined with lower blood pressure is a positive indicator. Using telemetry, you can monitor blood pressure over time and watch as the heart slowly fails. Early on you might have good compensation: walls might get thicker, you might see better function or higher blood pressure, but as the heart starts to fail those parameters drop. So we either use echo or blood pressure measurements to say with confidence that we have achieved heart failure.

6. **How might someone use telemetry to assess homogeneity of myocardial infarction?**

Dr. Tim Hacker: That's difficult. One of the challenges everyone wrestles with when looking at homogeneity of myocardial infarction is knowing when you have the same sized infarction. Surgically this is impossible,

because vessel anatomy is too variable. But we primarily use echo to get an idea of infarct size and a measure of function.

If you are less interested in the actual infarct size and more interested in whether or not an animal is actually going to go into heart failure, you can use telemetry to measure blood pressure. You can set a blood pressure threshold, below which you could deem heart failure and start treating it at that point. That could be a way to get homogeneity in a model that is notorious for not being able to control infarct sizes very well.

7. How does anaesthesia type and dose affect results acquired in unconscious animal models, whether using imaging systems, or other systems to assess cardiac function?

Dr. Tim Hacker: All anesthetics have an effect on cardiac function, so it is critical to titrate them accurately and consistently. As soon as an animal is put under anesthesia, it starts to lose body heat quickly (this is especially true for mice), so you need to have heated pads. As a mouse cools down, its heart rate drops and with a drop in heart rate you are going to have a change in chamber dimension. The goal is to replicate physiologic conditions as closely as possible with the anesthetic as low as possible. You also want to monitor heart rate to get an idea of the levels of anesthetic.

We tend to use isoflurane because it's easy. The mice go down and come up quickly, and it is gentle on them, so there is no risk of killing a mouse if it is diseased, for example. It does depress ejection fraction and heart rate, though. It is also a pulmonary vasodilator so if you're looking for changes in pulmonary hypertension, that is an important consideration. Ketamine depresses ejection fraction if the dose is high enough, and depresses heart rate further, but is less of a pulmonary vasodilator.

When we do terminal studies, our anesthetic of choice is urethane. We think urethane probably has the most minimal effect on cardiovascular function, but it is too long acting to use as a chemical restraint. Mice, especially if they're sick, won't wake up from urethane. When we use urethane, we get heart rates in the physiologic range, between 500–600 bpm and seem to have fewer effects on ejection fraction when given the right dose.

8. How would you select the most appropriate anaesthesia option to ensure your cardiac function results correlate well with data acquired in conscious animals using telemetry?

Dr. Tim Hacker: If you didn't have to wake up the animals, I would definitely use urethane. Urethane gets the closest: you're going to have the highest heart rates and similar breathing rates. Using other anesthetics cardiovascular function will be more depressed. That being said, we did a study where we looked at awake and anesthetized animals using isoflurane, and blood pressures in the anesthetized group was more similar to blood pressure in animals in a deep sleep than to anesthetized animals. Using other anesthetics, be aware that parameters will only match up during specific times of the day, probably when they're sleep rather than truly awake.

9. **When planning a study using a surgical model where the severity of the resulting disease may vary, (i.e. TAC where animals may ultimately progress to heart failure, while others may only develop hypertrophy) how do you handle this inherent variability in disease progression when testing new therapeutic compounds or treatment strategies?**

   Dr. Tim Hacker: That's tough. In TAC models, there's a lot of critical parameters. Most people just go by body weight and pick a wire size accordingly, but we've found that body weight isn't great. You can also try to pick animals that are all the same size. Sometimes if you're looking at transgenic animals and you compare them to wild-type animals, sometimes aortic sizes can be really different. We physically measure the aorta with calipers and choose a wire size to make a certain sized constriction based on that.

   The other way is to stratify models. Again, you can use telemetry to measure blood pressure, and watch blood pressure change over time and then pick heart failure at some pre-determined threshold. If you have a decently tight band, most of them will go to heart failure. Some might take a month, some might take 2 or 3, but that's a way to get around it as well.

   Scintica Instrumentation: The Indus Instruments Doppler Flow Velocity system may also be used to assess blood flow velocities through the carotid arteries, and the velocity of flow at the stenotic jet. The stenotic jet flow velocity can be used to approximate the change in pressure across the band; while the ratio between the left and right carotid peak flow velocity can be used to stratify the animals into a loose or tight band group. Further details can be found in the following references:

   Hartley, C. J., Ochoa, L. N., Reddy, A. K., Michael, L. H., Pocius, J. S., Pham, T. T., … Taffet, G. E. (2001). Vascular adaptations to transverse aortic banding in mice. Annual Reports of the Research Reactor Institute, Kyoto University, 1*(May 2014)*, 184–187.

   deAlmeida, A. C., van Oort, R. J., & Wehrens, X. H. T. (2010). Transverse aortic constriction in mice. Journal of Visualized Experiments : JoVE, (38).

10. **How important is surgical monitoring and the stability of the animal throughout the surgery (either during telemeter implantation, or surgery to cause cardiovascular disease), to the outcome of the overall study?**

    Dr. Tim Hacker: Animals are going to recover faster and you're going to increase survival rate if you have good surgical monitoring and stability. We use heated platforms with built-in ECG. They're also a big platform, so they're comfortable to use, so the surgeon can do get an implant in more quickly, and mice recover better and more consistently. In some cases, like with the ECG monitoring, you can also verify whether you actually have a myocardial infarction by looking at the characteristic ECG changes, like changes in the ST segments, to ensure you have the surgical model that you say you are creating.

11. Is there any dummy model where we can practice telemetry surgery in mice?

Dr. Tim Hacker: If you practice with a catheter (for BP implants) first, then you will be ready for the real thing. The ECG implants are straightforward.

Scintica Instrumentation: Depending on the configuration of a system to be purchased there may be an option to include a "dummy" telemeter for practice. This can be discussed in greater detail while discussing your specific research application.

12. Can you explain ultrasonic crystals usage?

Dr. Tim Hacker: Ultrasonic crystals are tiny crystals which can 'talk' to each other. They give information on their distance apart in real time. They can help to track regional changes of distances over time and thus can determine wall thickening, chamber diameter changes and regional wall motion and strain.

13. What is your opinion regarding normalizing heart weight to body weight or to tibia length? Depending on which normalization we choose, the interpretation about cardiac hypertrophy can vary dramatically.

Dr. Tim Hacker: In young animals (less than 4 months) generally body weight is fine, but once animals – especially males and some strains – put on fat which has much lower metabolic (and thus cardiac) requirements, the heart no longer grows in proportion to the body weight and thus tibia length becomes a better measure for normalization.

14. What is the best parameter to normalize one's data? Heart rate?

Dr. Tim Hacker: For structural measurements, LV mass, wall thickness, LV diameter etc by body weight. For functional measurements such as ejection fraction and fractional shortening, it's hard to normalize at all. Some functional measurements, such as isovolumic relaxation time, or time to peak AO pressure, or time based parameters, you can normalize by heart rate.

15. Have you done studies combining telemetry and echo?

Dr. Tim Hacker: No, but one could. In theory you could get pressure-volume data. We have used echo to measure pulmonary artery velocities and pressure at the same time.

See:

Wang, Z., Schreier, D. A., Abid H., Hacker T. A., & Chesler N. C. Pulmonary vascular collagen content, not cross-linking, contributes to right ventricular pulsatile afterload and overload in early pulmonary hypertension. *J Appl Physiol (1985)*. 2016;122(2):253-263.

16. **What is your advice for low cost pulmonary pressure measurement in acute experiments in rats?**

    Dr. Tim Hacker: Since the pressure differences are small, I would use a solid-state pressure catheter. A fluid filled pulled catheter attached to a pressure transducer and amp would also work and would be the least expensive option.

17. **What kind of functional readouts do you get by telemetry apart from more superficial readouts like heart rate? What are the applications and where are the limits?**

    Dr. Tim Hacker: For pressure, systolic and diastolic pressures, mean pressures. These can tell you the status of an animal's cardiovascular function and how it might change over time. For example, maybe you have a mouse with increased heart mass. If you measure blood pressure and it is elevated it may be the cause of the hypertrophy.

18. **What would be a good anesthetic for infarct size measurements in the mouse IR model?**

    Dr. Tim Hacker: Assuming this is a terminal event, as we measure MI size by histology, any will work.

19. **You mentioned cardiovascular changes with cage change and noise. Have you observed CV changes with other husbandry alterations such as rodent group size, bedding volume, enrichment, cage type, etc?**

    Dr. Tim Hacker: We have not tested group size, bedding volume or enrichment, except for a running wheel. A running wheel causes slight cardiac hypertrophy in mice. We have static cages and Innovive cages which have air running in and out of the cage for circulation. These cause a slightly higher BP in mice.

20. **Different mouse strains have different plasma elution patterns and different ratios of apolipoproteins – they respond differently to insult. Choosing one strain gives you a very small snapshot that may not be representative of a genetically varied population. Can we therefore conclude that studies using only one mouse strain is equivalent to looking at one individual? How do we draw any translational conclusions from one individual? Could this be a factor to why most therapies work in mice but the majority of clinical trials fail?**

    Dr. Tim Hacker: Without a doubt, mostly we use mice to screen and then also test in rats or rabbits or pigs to re-confirm.

22. **How would you analyze large chronic data sets? For example, is there a good auto-analysis software for telemetry ECG?**

    Dr. Tim Hacker: Most of the commercial analysis software can screen for events. Often they have parameters you can set (interval lengths and so on). There are also commercial services than will do the analysis for you.

23. **Let's say I have 4 groups and 10 animals for each group. Do I need to repeat the experiments, and how many times?**

    Dr. Tim Hacker: Hard to answer without seeing the data, but likely if the experiment is well done, once is enough. I would be more likely to repeat in another species.

24. **What is the best method to determine study size?**

    Dr. Tim Hacker: If you know the likely differences between groups and have some idea of your standard deviations, a power test will help to determine the study size. For mouse cardiovascular studies you'll need at least 10 animals per group, unless the differences are very large. Some types of studies like myocardial infarctions for example, where there is a larger variability, you may need a larger study size.

25. **What is a good laboratory where I can do short term training?**

    Dr. Tim Hacker: Excellent question. We offer a small animal surgery course several times per year:
    https://insidescientific.com/workshop/rodent-microsurgery-hemodynamic-measurements-training-program/

26. **How do you specifically and objectively define "perivascular region" for histological analyses?**

    Dr. Tim Hacker: For acute MI, we use TTC staining and Evan blue to determine the MI zone, the area at risk and area not at risk. We use this method:
    https://www.southalabama.edu/ishr/help/ttc/

    For chronic measurements, we follow this pub:
    https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2675697/

27. **Can we do renal artery implant under this condition?**

    Dr. Tim Hacker: Yes, although we have never tried it.

28. **I assess the homogeneity of myocardial infarction with ultrasonography on M-Mode Long axis and I weight scars after cardiomyocyte isolation. Which other parameter I can use to assess that?**

    Dr. Tim Hacker: We also slice and stain heart and put on slides as explained here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2675697/

29. **Are there any special considerations when planning methods for cardiovascular research in a pregnant mice model?**

    Dr. Tim Hacker: Mostly taking into consideration the timing of the plugging.

30. **Is the isoproterenol pump infusion model appropriate to study hypertrophy?**

    Dr. Tim Hacker: Yes, we have used it before and it works well, is consistent and easy to do.

31. **What is a mouse colony management strategy to generate enough male and female mice for experiments?**

    Dr. Tim Hacker: Have lots of breeders.

32. **Regarding HFrEF would you recommended using mice or rats? Which strain would you use for permanent ligature or ischemia/reperfusion?**

    Dr. Tim Hacker: Both will work well. We like Lewis rats as they will tolerate a larger MI and experience fewer arrhythmias, so our survival rate is higher. C57BL/6 mice are the mice we typically use for MI studies, but all will work. BALB/c mice tolerate a larger MI.

33. **Should one proceed to test for their proteins of interest to assess if a stable model has been attained, or rely on the assessment of gene expression to ascertain whether a stable model has been achieved?**

    Dr. Tim Hacker: The proteins of interest are likely better as gene expression could give a false sense of change, since the turnover of the protein may be altered. For example, you could have high gene expression and actually increase protein production, but also very high degradation so the net of amount of protein may be unchanged or even lower.

34. **When employing telemetry for measurements such as ECG or voluntary exercise, do I need to monitor all the animals?**

    Dr. Tim Hacker: One advantage of telemetry is the need for fewer animals, so you can use a subset of animals to prove your point. We have found with voluntary exercise, not all animals will run, so in the case of exercise, I would monitor all animals.

If you have additional questions for Scintica Instrumentation regarding content from this webinar or wish to receive additional information about their chronic cardiovascular instrumentation, please contact them by phone or email:

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