Unanesthetized Sublingual Blood Collection in Rats
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Research History:

Working for a contract research laboratory, we get many requests from clients for specific procedures that we have not yet encountered. One of these specific procedures was sublingual blood collection in rats. Therefore, we were tasked to research this procedure and develop it within our own facility. When researching this procedure we found two main articles that were instrumental in the development of this procedure.

In 1998 and 2000, Novartis out of Basel, Switzerland published two articles referencing Sublingual Blood Collection in Rats. In these articles, they referenced initial documentation of this procedure dating back to 1964, in which the procedure was performed on an unanesthetized rat, however they used eye scissors to sever the vein and get blood. This was not a favorable method, due to damage to the tongue, including swelling. Novartis further refined this procedure. First they would anesthetize the rat. They then utilized two technicians: one to hold the rat by the scruff, the other would extend the tongue and puncture the vein using a 23 gauge needle, instead of severing the vein. In this procedure they sighted no adverse effects on body weight, clinical observations or food consumption. They also found that this could be used for repeated sampling, up to 7 time points, with minimal damage to the tongue, as the tissue of the tongue heals quickly. Further research on the technique showed comparable blood parameters to other methods such as orbital sinus (retrobulbar plexus), not to mention it had less tissue damage than orbital sinus.

Though this procedure seemed successful for Novartis, it seemed to be somewhat of a cumbersome process for our facility in comparison to other blood collection methods. In order to get this procedure developed in our facility, our IACUC, study directors, and management had a list of things they wanted to be considered to make this viable for use. For example: 1) is it a reliable method, 2) can it be performed unanesthetized, 3) can it yield high volume, 4) is it easy to train, 5) how can we reduce contaminants in the mouth, and 6) overall, it would have to be an improvement upon current blood collection methods already utilized in our facility. Within these guidelines, the following blood collection was developed.

Procedure:

1) First the rat will be placed in a gavage hold in which the head is back, and the corners of the mouth are drawn back (almost as if you are making the rat smile), which will extend the tongue so that the sublingual veins are visible and the rat is securely, but comfortably restrained. Note: This hold is very important for this procedure. If the hold is not done properly, blood collection and quality could be compromised. Some common issues we find with this hold are a tendency to squeeze around the thoracic region, holding the rat against the palm of the hand. If the chest cannot expand during respiration, then the rat will struggle.

2) The mouth will then be rinsed with tap water/saline (optional) to remove any contaminants, such as food particles or excess saliva. Be sure to do this with the rat inverted to avoid aspiration. Note: This step is not necessarily needed, but if done, best practice is to use saline for a rinse as opposed to water. However, if the tongue is not dried appropriately, and water or saline gets into the sample the water may lyse the blood cells, whereas the saline would not.

3) The tongue is then dabbed dry using a piece of gauze. Note (very important): This step is very important whether you use a rinse or not, as the saline, water, or saliva could combine with the blood in the tube, and contaminate or lyse the blood cells causing hemolysis.

4) Using a 25g needle (a 23g needle may be used if there is not sufficient blood flow, if a large volume is required), puncture the vein in a direction parallel to the vein with the bevel up. If repeated sampling is required, puncture proximal to the previous site, or alternate left and right sublingual veins. Note: You will almost always get blood.
However, if it does not bleed readily, then you may have poked just the tip of the vein, or the tongue itself. You also do not want to poke at the very base of the tongue as in our experience it could create a hemATOMA. The middle or the meat of the vein is the best practice.

5) Due to the amount of blood pressure to the head, blood will immediately and abundantly flow. Invert the rat slightly, but not so much that the nose is facing down completely, and allow the blood to drip from the puncture site directly into the collection tube. Note: We have found that if any blood gets on top of the tongue, or if the mouth is not held open correctly and blood gets on top of the tongue, the tongue will form a seal with the top of the mouth creating a little reservoir for the blood to pool and not drip from the mouth. Also, if blood gets on top of the tongue, the rat will want to lick, which it cannot do, so it will react and squirm.

6) Then, using a piece of gauze or a cotton tipped swab, pressure will be applied to the puncture site to arrest blood flow. Note: If using gauze, use your finger to put pressure on the tongue, and push back into the corner of the mouth so that your finger acts as a bite bar. With this, the animal will not be able to bite down if it were to try. Also, your finger will be behind the teeth, so if they manage to bite down it will go around your finger and you cannot get bit. Another method for arresting blood flow is to pack gauze on the side of the puncture site and hold it there to achieve hemostasis. Hold the pressure for about 30 to 45 seconds without checking the wound so that it has time to close. Check the rat for a few seconds to make sure blood is not pooling under the tongue. If it is not pooling, then the animal is done bleeding. Also, it is advisable to hold the animal is such a way that if blood flow is not arrested quickly, and blood is pooled in the mouth the rat cannot aspirate. Note: When checking make sure not to open the mouth completely, as this could reopen the wound and it may start bleeding again.

Conclusions:
In our experience, we can perform this procedure with two people, one to do the blood collection, one to run rats and hold off the tongue. This can be performed at a rate of approximately 1 animal per minute if doing a timed toxicokinetic or pharmacokinetic blood collection, and can yield up to at least 3 mLs if performing this for hematology and chemistry parameters.

Pros and cons of this procedure compared to other bleed methods, i.e.: orbital sinus, tail vein and jugular vein are listed below.

Pros:

- It can be performed unanesthetized
- No warming cabinet or water bath is needed
- It uses minimal restraint
- It is easy to train
- The vein is visual, no blind sticks, reliability
- It can yield high volume
- It can be performed bilaterally
- It can be used for repeated blood sampling
- Heals quickly, minimal tissue damage
- Minimal risk to the health of the animal

Cons:

- In some instances, when using a larger needle, it may take more time to arrest blood flow
- Because it is a fairly new procedure, historical data is minimal, and it is not widely used
- If one of the chemistry parameters is Amylase, for obvious reasons this parameter may be off.

In conclusion, unanesthetized sublingual blood collection, if performed correctly, seems to be a win-win situation for the process of collecting blood with minimal discomfort or risk to the animal.

References:
2) “Comparison of clinical pathology parameters with two different blood sampling techniques in rats: retrobulbar plexus versus sublingual vein”, Andreas Mahl, Peter Heining, Peter Ulrich, Josef Jakubowski, Maria Bobodilla, Walter Zeller, Reinhard Bergmann, Thomas Singer & Lothar Meister Novartis Pharma AG, Preclinical Safety: Toxicology/Pathology, CH-4002 Basel, Switzerland. Laboratory Animals Ltd. Laboratory Animals (2000) 34, 351-361

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