Refinement Recommendations for Intravenous Injection and Body Heat Conservation in Mongolian Gerbils used in SPECT Studies

by ***Christel Palmyre Henri Moons[1,*], Kathelijne Peremans[2], Philippe Büscher[3], Simon Vermeire[2], Eva Vandermeulen[2], Kurt Audenaert[4], Katleen Hermans[5] & Frank Olof Ödberg[1]***

[1]Laboratory for Ethology, Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

[2] Department of Medical Imaging of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

[3]Parasite Diagnostics Unit, Department of Parasitology, Institute of Tropical Medicine Antwerp, Antwerp, Belgium

[4]Department of Psychiatry and Medical Psychology, Faculty of Medicine, Ghent University, Ghent, Belgium

[5]Department of Pathology, bacteriology and poultry diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Summary

This paper describes two aspects of Single Photon Emission Computed Tomography (SPECT) which have been refined for use in small animals, as demonstrated by a study on Mongolian gerbils: intravenous injection of radiotracers in the femoral vein and body heat conservation in animals anaesthetized at the time of scanning. Elaborating on recommendations made by other authors, we have combined chemical and physical restraint to optimize injection efficiency as well as to reduce handling time of the animals. Finally, we provide a detailed description of a SPECT-compatible water-heated scanning bed, the application of which we believe is not limited to scintigraphic studies.

Introduction

SPECT allows functional imaging of biochemical molecules when an injected radioactive ligand in a living animal binds to a specific target (receptor, transporter or enzyme). Already widely used in human and large animal studies, the technique is increasingly gaining interest among scientists studying small laboratory animals given the minimally invasive nature of the procedure and the resulting opportunity to perform longitudinal studies (*Peremans et* al*., 2005*). The purpose of this paper is to elaborate on the refinements we applied to the intravenous injection technique and body heat conservation in Mongolian gerbils used for SPECT studies (*Moons et al., 2008*), but which we believe also to be useful in other types of research involving small laboratory animals.

**Correspondence:* Christel Moons
Laboratory for Ethology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
Tel. +32 9 264 78 09
Fax +32 9 264 78 49
E-mail christel.moons@ugent.be

First, injecting the radioactive tracer directly into the bloodstream constitutes a technical challenge and the entire procedure should be as refined as much as possible to minimize animal discomfort. Much of the same considerations for blood sampling must be given when injecting a substance (*Morton et al., 1993*): the site of injection, the volume of injected substance, the type of needle and syringe used, and the least-stressful handling methods. The femoral vein has been reported as a suitable site in gerbils for intravenous injection of substances by some authors (*Perez-Garcia et al., 2003*) while others recommended the jugular vein (*Palm & Hollander, 2007*). The latter reported injected volumes of up

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to 0.5 ml whereas the former injected 0.1-0.2 ml, but it is not stated whether this is the maximum volume possible. However, both methods described consist of a surgical procedure, which involves making a skin incision, which must be sutured to close. Although indicated to be short procedures (unspecified length by Perez-Garcia et al. (*2003*) and "less than 5 min" by Palm & Höllander (*2007*)), both methods involve post-injection manipulation of the animal, which means the start of a SPECT-scan is postponed and the scientist is additionally exposed to radiation at close range.

Second, animals have to be immobilized during SPECT to minimize blurring of the scanning image and optimize spatial resolution. From a practical point of view physical restraint -especially in gerbils and hamsters- is inferior to chemical immobilization using anaesthetics *(Hem et al., 1998)*. It is true that both can have an effect on physiological parameters as well as tracer uptake properties, but the effects of anaesthetics can be standardized by using the same anaesthetic protocol throughout a study (*Peremans et al., 2005*). Animals are especially prone to body heat loss during anaesthesia and a key factor is the surface to body mass ratio, which is considerably smaller in small animals, causing them to lose heat more rapidly. In dogs, hypothermia appears to be associated with prolonged recovery (*Pottie et al., 2007*) and also in humans there are a number of negative effects linked to a decrease in body temperature during anaesthesia (*Pannen, 2007*). In rodents, hypothermia is even deemed to be one of the most important causes of death during anaesthesia (*Hanusch et al., 2007*). Therefore, to ensure recovery and minimize discomfort in all species, but especially those of smaller size, body temperature should be artificially maintained and, if possible, actively monitored. The heat source is best applied from the start of the procedure, as it is more challenging to re-warm an individual afterwards than to prevent heat loss during anaesthesia (*Weyland et al., 1994*). Both in humans (*Leung et al., 2007; Sury & Scuplak, 2006*) and animals (*Tan et al., 2004; Weinandy et al., 2005*), a heat source is more efficient in preventing heat loss when it surrounds the anaesthetized individual rather than when it is placed underneath. Water-driven heating sources are preferred over electrical heating pads as the latter risk causing severe burns

Materials and Methods

Prior to the experiment, approval for the procedures from the Ethical Committee of the Faculty of Veterinary Medicine at Ghent University was obtained. The Laboratory for Ethology is licensed for breeding and carrying out experimental procedures on gerbils (LA2400377; LA1400096).

Animals

The study comprised nine male Mongolian gerbils (*Meriones unguiculatus*) aged 378 ± 63 days. Our subjects were bred from four pairs of Mongolian gerbils purchased from the outbred SPF RjTub:MON stock from Elevage Janvier (Le Genest-St-Isle, France). The animals were group-housed (2-3 animals per cage) in Makrolon type IV cages (55 x 33 x 20 cm, Bio-Services, Schaijk, The Netherlands) in the colony room at the department of Animal Nutrition, Genetics, Breeding and Ethology. The cages were bedded with Gold Mix wood shavings (Carfil, Turnhout, Belgium) and cage enrichment was provided in the form of Mini-Tork™ paper tissue (Tork, Guildford, Australia), hay, and chew blocks. The chew blocks were homemade by cutting and dividing branches from apple and cherry trees. Pellets of 2016 Teklad Global 16 % protein rodent diet (Harlan, Horst, The Netherlands) and tap water were available *ad libitum*. All animals were kept under a 14:10 light:dark cycle with lights off between 9:00 and 19:00. Room temperature averaged 20 ± 1 °C whereas relative humidity values ranged between 30 % and 60 %. Continuous ventilation was provided.

Intravenous injection

Animals were briefly anaesthetized in an induction box using isoflurane (4% induction) and the fur

of the medial aspect of the hind leg was clipped (Wella, Bio-Services, Schaijk, The Netherlands). Next, the gerbil was placed inside the restrainer in dorsal recumbency. The tail was fixated between middle and index finger while one hind leg was held between thumb and index finger. Both tail and hind leg were extended through the horizontal part of a T-shaped hole of a Plexiglas plate that was placed between the restrainer tube and two Plexiglas protrudings. Care had to be taken that fixation pressure was applied most to the tail rather than the hind leg to avoid dislocating any joints. Lifting or lowering the leg ensured soft clamping and release on the vein, respectively. A slightly bulging vein is easier to identify and access. Figure 1 shows the positioning of the gerbil at the time of injection.

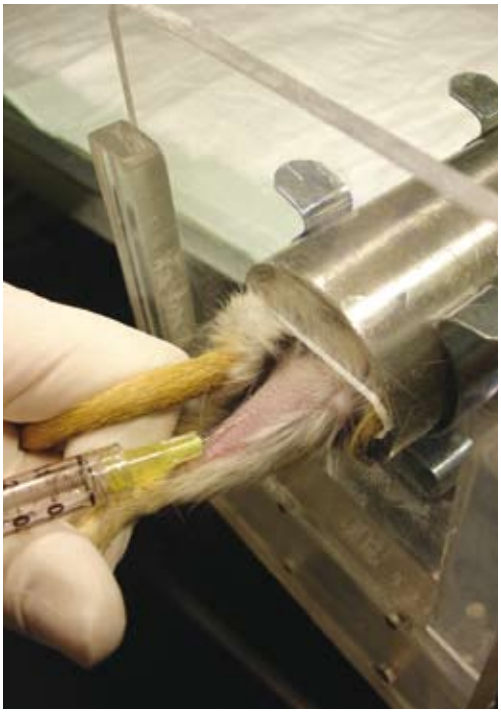

Figure 1. Position of gerbil in restrainer during femoral vein injection of radioactive tracer. A low-resistance syringe is fitted with a 30G bent needle held perpendicular to the vein prior to insertion.

The tracer was injected at body temperature (38°C; (*Mele, 1972*)) in the femoral vein using a BD 1 ml syringe with Luer-Lok™ tip (BD, Temse, Belgium) fitted with a slightly bent 30 G needle (*Perez-Garcia et al., 2003*) that was guided parallel along the vein after subcutaneous puncture and prior to venous puncture. In our experience, the BD syringe offered much less initial drag when pressing down the plunger as compared to e.g., a Terumo Myjector® syringe and allowed for smoother handling of the plunger, thereby decreasing the risk of vessel damage. We found that it is feasible to inject up to 0.6 ml in the femoral vein of male adult gerbils, although this amount of fluid is not necessary if the radioactive tracer is less diluted. The reported circulating blood volume of an adult gerbil is 67 ml/kg and it has been recommended not to inject more than 10% or 0.67 ml for a gerbil of 100 g in one single intravenous injection (*Diehl et al., 2001*). Because of the relatively large volume in comparison to the animal's blood volume, we emphasize the importance of heating the fluids prior to injecting.

The combined use of the restraining device and anaesthetic optimized successful injection of the fluid into the tiny vein and improved ease of handling of the animal, thereby reducing the duration of the acute stressor. First, a fully conscious gerbil is difficult to restrain and fixating the tail and the leg may result in injuries. Second, because the animal tenses its muscles and experiences acute stress, the blood pressure within the vessel increases. Consequently, after retraction of the needle the risk of prolonged bleeding and haematoma formation becomes substantial. On the other hand, the veins of an animal under general anaesthesia are subject to vasoconstriction and become more difficult to puncture. Therefore, the time of injection was carefully chosen to coincide with the point where the gerbil started to regain consciousness, but at that moment was not yet providing so much resistance that fixating its tail and leg could cause injuries. The compression time to close the puncture wound was between 30 sec and 3 min. After injection, compression of the injection site, and verification

that no haematoma was forming, the animal was placed back in its home cage where radiation heat continued to be provided using an infrared lamp (200W). The interval between the moment the gerbil was anaesthetized and when it was returned to its home cage, was less than 5 minutes.

Heated scanning bed

Prior to scanning, the animals were again placed in the induction box and anaesthetized with isoflurane (4% induction, 1.8 % maintenance). Once anaesthetized, they were transferred to the scanning bed and placed in sternal recumbency. Because the animals were prone to heat loss throughout the 20-minute scan, we adapted a previous version of the scanning bed to include an apparatus based on circulating warm water (38°C) to promote post-anaesthesia recovery by minimizing body heat loss (Figure 2). A PVC tube (∅ 5 cm) was placed inside another PVC tube (∅ 6 cm) and both were glued together at the top. As a result, space was available between the two tubes for heated water to flow through. The rear part was sealed using concentric pieces of PVC and silicon glue and a smaller PVC tube ensured attachment to the scanning bed holder. Towards the front end, parts of the 'dorsolateral' sides of both tubes were cut, allowing visual respiratory monitoring of an anaesthetized gerbil. The space in which water could circulate spanned the entire gerbil body, but excluded the head in order to avoid attenuation of the photons and therefore interfere with proper detection of radiation. Two tubular PVC connections (∅ 1 cm) were glued to holes cut in the bottom of the outer PVC tube and were connected to 2 pieces of flexible plastic aquarium tubing, one for inflow and one for outflow of water. An aquarium pump (Eheim, type 2011, Deizisau, Germany) was attached to the inflow tubing and pumped water through the scanning bed from a temperature-controlled water bath at 4.5 l/min, which was positioned underneath the table to which the scanning bed was attached (Figure 3). The incoming water was guided to the front end of the scanning bed by a U-shaped PVC

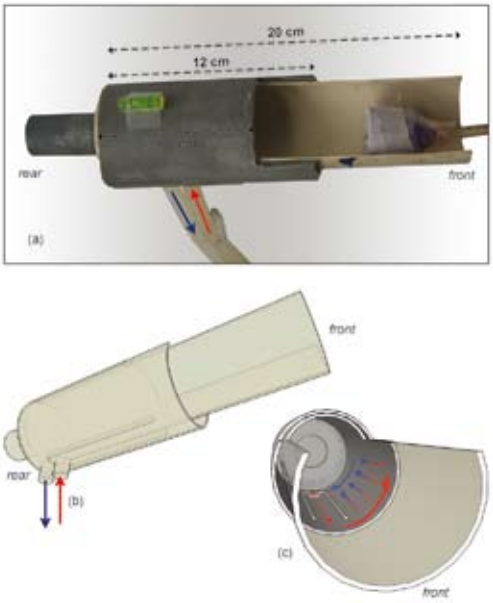

Figure 2. (a) Dorsal view of apparatus to prevent heat loss in anaesthetized gerbils. (b) Schematic ventral view of apparatus. (c) Schematic frontal view of apparatus. Arrows indicate water of 38 °C flowing between the two tubes (red: inflowing, blue: outflowing).

Figure 3. (a) Overview of SPECT scan setup. Triple head gamma cameras circle the water-based heated scanning bed. (b) water bath with aquariumpump for water circulation.

insertion between the two layers of the PVC tubes. The scanning bed was fixed to a stand that had been constructed previously for other small animal studies. Two water gauges (one on the stand and one on the apparatus) ensured horizontal placement of the scanning bed between the SPECT camera heads.

Discussion

As argued by other authors (*Peremans et al., 2005*), a technical challenge encountered during a procedure involving SPECT and scintigraphy in general is the intravenous injection in small animals. During pre-trials, we initially tested the procedure of surgically exposing the femoral vein via an incision and blunt dissection as proposed by Pérez-García (*2003*), but since the femoral vein in the gerbil is visible subcutaneously when the fur is removed, we found that the method gave us no efficiency or time advantage over performing direct venepuncture through the skin. Our method as compared to that of Palm & Höllander (*2007*) yielded the same injectable volume and at what we consider to be a less critical injection site. Furthermore, it is our opinion that a skin incision is a contraindication when radioactive material is injected because it can interfere with the experimental timeline and increase the radiation burden on the scientist. Radiosafety implies minimizing contact between the radioactive substance and the environment whenever possible and a sutured wound is a risk factor should subcutaneous bleeding (bruising; (*Morton et al., 1993*)) occur at the time of venipuncture. However, we are convinced that in studies other than those involving injection of radioactive substances, both methods are valuable and refined tools for intravenous administration. In our study, training sessions preceding the actual SPECT experiments, in which intravenous femoral vein injection (0.9 % NaCl) was practised 20 times (left and right femoral vein of ten animals), resulted in a high success rate when injecting the radioactive substances. Out of nine animals used in the study described here, intravenous injection failed in only one on the first try, but was subsequently successful when injecting into the femoral vein of the other hind limb.

Second, the body of a small animal has a larger surface area to mass ratio than that of a larger animal and therefore it is more prone to hypothermia. The continuous supply of heat energy from the water-based device we constructed was intended to prevent body heat loss in order to avoid additional discomfort as well as mortality in the gerbils. Although the NaI detector crystal is hygroscopic and absorption of water could lead to discoloration and reduced detection capability (*Saha, 2006*), there is no actual risk in using a water-based apparatus as the crystal is sealed in aluminium containers and the layers of the scanning bed are carefully sealed with silicone glue. All of our animals survived the SPECT procedure. No temperature measurements were taken during the SPECT scans because handling the animal would interfere with the quality of the images. Monitoring body temperature would be possible using implantable telemetry, but in this study the detection infrastructure required for body temperature recording could not be combined with the SPECT equipment. However, the body and extremities of all animals felt warm when the gerbils were returned to their home cage, where heat continued to be supplied by an infrared lamp. The use of isoflurane allowed for precise depth of anaesthesia control and the induction box reduced handling stress. In gerbils, recovery after isoflurane, as opposed to an injectable anaesthetic such as pentobarbital, is quick with the animals moving almost immediately after an anaesthetic procedure is terminated (*Weinandy et al., 2005*). Although there may not be extreme hypothermia, Weinandy et al. demonstrated that in gerbils, body temperature normalises only after 7-8 hours. This implies that, during recovery, elimination of the drug is a more important determining factor than motor activity to compensate any body heat loss.

Conclusion

We have introduced two methods of refinement for small animals used in SPECT regarding intravenous injection of substances into the femoral vein and preventing body heat loss during scanning. Combining chemical (isoflurane) and physical restraint and a carefully timed moment of injection, proved to be an efficient technique. Another recommendation is the use of a syringe with little or no drag on the plunger to increase smooth manipulation and reducing the risk of vessel damage. Using these methods, we were able to successfully inject a substance into the femoral vein of Mongolian gerbils. Furthermore, we have presented detailed information about a water-based heating device/scanning bed which was custom designed for use with the SPECT imaging equipment. The heat that surrounded the animals during the 20-minute scans, was aimed at preventing a decrease in body temperature that might threaten the animal's survival.

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