GV-SOLAS
Gesellschaft für Versuchstierkunde
Society for Laboratory Animal Science

Opinion

from the Committee for Animal Welfare Officers

Applications into the retrobulbar venous plexus in mice

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Introduction

In its expert information on substance administration in laboratory animals, the Committee took a critical look at the usual methods of administration.

Several recent licensing applications under § 8 para. 1 of the German Animal Welfare Act have stated that cell suspensions were to be administered intravenously to mice not via the lateral tail veins, as is generally the case, but via the retrobulbar venous plexus. The applications cite a publication by Hall et al. (2007), in which the authors injected haematopoietic stem cells via both routes for comparison and found that injection into the retrobulbar venous plexus led to significantly higher transplantation success rates, higher transplanted cell numbers and lower variability compared with intravenous injection into the lateral tail veins.

Evaluation of the method

Injection into the retro-orbital venous plexus is not a standard method of parenteral administration and is not recommended as a standard method by GV-SOLAS for the following reasons:

- Injection into the retrobulbar venous plexus is assumed to cause more, and more traumatic, local changes than a puncture at this site for blood collection: the injection is an active application, and it allows the sharp injection needle to move freely in the tissue.
- Intravenous administration is not guaranteed, as the method is performed blindly into a sinus and correct needle position cannot be verified visually.
- The volume actually administered intravascularly cannot be reliably determined.
- The proportion that is injected extravascularly can cause local tissue irritation if the properties of the solution/suspension/emulsion are unfavourable, and exophthalmos if the volume is too large.

The authors are currently unaware of any scientific purpose for which retrobulbar administration is indispensable. If the method is nevertheless deemed essential in particular cases, the following points should be taken into consideration:

- The method may only be performed by specially trained persons with demonstrable qualifications.
- It may only be performed under anaesthesia (if necessary, general anaesthesia may be supplemented with local analgesia using eye drops or ointment).
- The injection must be performed slowly.
- Needles of gauge 27G or thinner should be used.
- No irritants or solvents may be used.
- No tumour cells may be used.
- Only single-cell suspensions may be used.
- A maximum of 0.15 ml per 30 g BW may be administered in an adult mouse.
- Only one procedure per day is permitted, for a total of two procedures per eye with a minimum of two weeks between procedures.

The method places higher demands on the skills of the person carrying it out, notably higher than the standard method of administration into the tail vein. Although the available literature (see below) shows no clear evidence of traumatic changes in the eye, this can initially be taken only as an indication that these published studies were conducted by especially well-trained personnel and that no data with negative consequences have been published as yet.

It is difficult to estimate the impact of this intervention on the animals, as this is particularly dependent on the individual skills of the person carrying out the procedure. Provided the procedure is performed correctly and in compliance with the above conditions, the stress caused can currently be assessed as low.

Summary of the literature on this method

The method of administration via the retrobulbar venous plexus is not new in itself. It was first mentioned by Pettit (1913: cited according to Tilgner & Metzke 1964). It is also described in a modern handbook of laboratory animal science (Pekow & Baumans 2003).

In the hands of a skilled operator, administration via the retrobulbar venous plexus is considered to be sufficiently safe and comparable to the usual method of administration via the lateral tail vein. The pathohistology data is not consistent: both the occurrence of necrosis and the absence of tissue trauma have been described.

As possible complications, the risk of breaking through into the arterial bloodstream if high or excessive pressure is used, as well as the expected introduction of some of the injection solution or suspension into the surrounding tissue is stated in the literature. Colonisation of surrounding tissue by tumour cells during tumour cell injections is seen as a particularly serious risk.

The main advantage cited for retrobulbar administration is the reduced stress for the animals. However, the stress depends on the type of restraint: for retrobulbar administration the animals should generally be placed under anaesthesia, whereas for tail vein administration they are restrained for several minutes during the procedure itself and also while the tails are warmed up. It is also argued that there are repeated failures in tail vein application that would require the use of additional animals.

Closely linked to this is the reduced time required: for retrobulbar administration, anaesthesia plus injection takes around one minute; for administration into the tail vein, tail warming alone takes several minutes.

A further advantage is that retrobulbar application is also possible in animals with heavily pigmented or scarred tails. It can also be used in newborn mice, hamsters, and guinea pigs, in which tail vein administration is not possible.

Literature in chronological order

Tilgner S., Metzke H. (1964), in GERMAN:

- Detailed description of anatomical conditions in rabbits, guinea pigs, golden hamsters, rats, mice and steppe lemmings;
- general description of orbital vein administration in these species.

Pinkerton & Webber (1964):

- Administration of 0.2 ml of contrast medium; anaesthesia not generally required;
- warning of excessive pressure, then injection into the arterial circulation and bone fractures possible;
- recommended for mice, hamsters, guinea pigs and rats, technically more difficult than retro-orbital blood collection as larger vessels need to be found;
- advantages: no warming of animals, faster, less stress;
- has advantages in animals with black tail pigmentation or without tails (hamsters, guinea pigs).

Voelcker & Fortmeyer (1979), in GERMAN:

- No difference in excretion kinetics of inulin following retro-orbital or tail vein administration;
- volumes up to 10 ml/kg BW possible;
- use of size 27G needles;
- no information on anaesthesia;
- method is considered applicable for mouse, rat, hamster and guinea pig.

Weisbrod (1982), in GERMAN:

- Use in mice and rats in toxicity tests;
- use of size 25G needles;
- maximum possible volumes in the mouse 8–10 ml/kg BW; in the rat 4–5 ml/kg BW;
- always under anaesthesia;
- method has particular advantages for repeated injections and for tail injuries in male mice kept in groups;
- for rats, no advantage is seen over tail vein injection.

Price et al. (1984):

- Single administration of radiolabelled reagents, thymocytes and B16 melanoma cells;
- volumes 0.2 ml using size 27G needles;
- no difference in excretion and distribution between the two administration methods after 12 h;
- but warning of colonisation by melanoma cells in orbit and brain and death of animals in a preliminary trial;
- advantages: no need to warm animals to dilate the tail veins, hence faster administration, less stress, especially suitable for animals with black tail pigmentation.

Hall et al. (2007):

- Application of humane hematopoietic stem cells in sublethally irradiated recipient mice comparing both application routes;
- volumes 0,030 ml;
- injections into the retrobulbar venous plexus led to significantly higher transplantation success rates, higher transplanted cell numbers and a lower scattering of results compared to intravenous injection into the lateral tail veins.

Nervi et al. (2007):

- Application of humane T cells in sublethally irradiated recipient mice comparing both application routes;
- volumes 0,2 ml;
- tail vein application of 10 x 10^6 cells caused only small and temporary engraftment of the transplanted humane T cells and no lethal Graft-versus-host disease in contrast to the retrobulbar application of the same amount of cells. The authors attribute the difference to the fact that the cells are retained in the retrobulbar sinus after retrobulbar application, multiply there and then spread via the local lymph vessels - in contrast to intravenous application, in which the cells are transported directly to the lungs.

Steel (2008):

- Daily administration of a test substance over five days: retrobulbar administration under isoflurane anaesthesia with daily change of eye in comparison with tail vein administration without anaesthesia and with manual fixation of the tail through the cage lid, warming with 50 Watt lamp for 2–3 min followed by injection into the lateral tail vein;
- administration of 0.08 ml, no information on needle size;
- comparative study of stress responses;
- no detectable differences in substance effect;
- advantages: histological evidence of local inflammation but no other traumatic damage at the injection site;
- faster administration;
- no difference found when studying pharmacological effects;
- higher aggressiveness in animals in the tail vein group.

Schoell (2009):

- Comparing the administration of 1 ml of a 10:1 mixture of ketamine (100 mg/ml) and xylazine (100 mg/ml) by the retro-orbital or tail vein route (needle size 26G);
- in the retro-orbital group the animals died 5 sec., in the tail vein group 3 sec. after injection;
- taking preparation time into account, the figures were 10 sec. and 60 sec. (not including time under the heat lamp);
- suitable for special studies in which tight kinetics need to be determined and mechanical killing methods are not an option.

Yardeni et al. (2011):

- Detailed description of the methods for adult (Isoflurane anaesthesia and additional local anaesthesia; needle size 27G or smaller, max. volume 0.15 ml) and neonate mice (without anaesthesia, needle size 31G, max. volume 0.01 ml);
- training of the surgeon necessary;
- only single cell suspensions, preferably body warm;
- histological changes have never been detected, but occasionally the leakage of injected material into the surrounding tissue, which is why the application of tumour cells is not recommended.

Schoch A. et al. (2014):

- Comparison of blood levels after application of a monoclonal antibody (volumes between 0.160 und 0.280 ml) under anaesthesia in comparison to application into the lateral tail vein;
- measures of the pharmacokinetic study show no differences between application routes;
- no clinical symptoms were observed after application, the number of misapplications after application into the tail vein required the replacement of additional animals;
- when complying with the GV-SOLAS recommendations, the retrobulbar application is a valid alternative to tail vein applications, although the recommended volumes were sometimes clearly exceeded.

Socher et al. (2014):

- application of 0.02 ml;
- Contrast agent under anaesthesia via both application routes, the animals were killed under anaesthesia after the measurements;
- visualisation of the heart and lungs was possible after retrobulbar application because there was no dilution of the contrast medium by other vessels in the inflow area of the caudal vena cava.

Leon-Rico D. et al. (2015):

- application of hematopoietic stem cells comparing both application routes;
- volumes 0.2 ml;
- injection into the retrobulbar venous plexus led to comparable transplantation success compared to intravenous injection into the lateral caudal veins, with less scattering of results. The authors emphasise the simplicity and speed of the method, which eliminates the disadvantage of unpleasant handling of the eye.

Bohnert (2019):

- comparative retrobulbar and tail vein application of doxorubicin, a locally irritating substance, to induce nephrotic syndrome, via catheter 0.2 ml, under anaesthesia;
- for histopathological analysis of the injection site, 5 animals each were killed 5 days after retroorbital injection of doxorubicin or physiological saline solution and 5 animals 25 days after doxorubicin application, as well as 1 animal 10 days after tail vein injection of physiological saline solution and 9 animals after doxorubicin;
- histopathological changes after retrobulbar and tail vein application were comparable.

Other information on retro-orbital administration

The Institutional Animal Care and Use Committees (IACUCs) of a number of US universities (e.g. University of California, Oregon University, New York University) regard the method for tissue-compatible substances as an acceptable alternative to tail vein administration. They limit the volume administered to 0.1–0.2 ml per eye and adult mouse, to one procedure per day and a total of two procedures per eye with an interval of 1–2 days (California, New York) or 2 weeks (Oregon) between procedures.

Administration should be performed slowly and under anaesthesia.

University of Arizona:
https://researchintegrity.asu.edu/sites/default/files/2018-05/SIG-Retro-Orbital-Injections-in-Mice-2-28-17.pdf

University of California:
https://iacuc.ucsf.edu/sites/g/files/tkssra751/f/wysiwyg/STD%20PROCEDURE%20-%20Misc%20Rodent%20Procedures%20-%20Retro%20Orbital%20Injection%20in%20Mice.pdf

Michigan State University: https://animalcare.msu.edu/guidelines/IG043.pdf

The University of Pennsylvania offers a video for training:
https://www.research.psu.edu/animalresourceprogram/training/videos/retro-orbital-injection-in-the-mouse

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Steel CD, Stephens AL, Hahto SM, Singletary SJ, Ciavarra RP. 2008. Comparison of the lateral tail vein and the retro-orbital venous sinus as routes of intravenous drug delivery in a transgenic mouse model. Lab Anim 37:26-32.

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