Blood Sampling from the Orbital Sinus

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Abstract

Blood sampling from the orbital sinus of rats is important for time course in vivo preclinical experiments.

Reagents

• Halothane (prescription is needed; Halocarbon Laboratories, River Edge, NJ USA), and a glass desiccator for halothane anesthesia.

• Fresh D.I. water

Equipment

• Hood

• Surgery desk

• Surgical operation boards (6) wrapped with absorbent underpads (Rolls, 20’x100’; Catalog No. 14-206-64; Fisher Scientific, Pittsburgh, PA USA)

• High intensity surgery light (Model 11201; Burton Medical Products Corporation, Van Nuys, CA USA)

• Fan (Generic brand)

• Kimwipes paper (Catalog No. 06-666A; Fisher Scientific)

• Standard haparinzed hematocrit capillary tubes (color code: red, haparinized with 2 U.S.P. units of ammonium heparin, 75 mm length, 1.1-1.2 mm i.d., 0.2 mm wall thickness; Catalog No. 21-176-6; Fisher Scientific)

• Flat-top Snap-cap microcentrifuge tubes (11 o.d. x 40.6 l. mm.; Catalog No. 05-408-25A; Fisher Scientific, Pittsburgh, PA USA, 1-800-766-7000)

• Centrifuge (Brinkmann Instruments, Inc., Eppendorf Centrifuge 5415C, Westbury, NY USA)

Procedure

1. Fast the rat for 16 h.
2. Use thick gloves to take the rat out of cage and put it into a container with a lid. Put the rat into a glass desiccator with vaporized halothane inside under the hood. Watch carefully not to over-anesthetize the rat.
3. After the breathing rate of the rat slows down, take out the rat from the desiccator, and lay it on its ventral surface with its tail 90 degree towards the investigator on the operating board.

4. By pressing down with the thumb and forefinger just behind the eye and pulling back the skin, the eyeballs are made to protrude.
5. A haparinzed hematocrit capillary tube (35 mm long) is positioned at the inner corner of the eye, beside the eyeball.
6. Then, the tube (approx. 45 degree angle to the eye) is slid about 5 mm forward gently by twisting but firmly along the side of the orbit to the ophthalmic venous plexus (orbital sinus) which lies at the back of the orbit. The blood vessels of the orbital sinus are extremely fragile and rupture on contact with the tube tip, allowing blood to drip into a collecting tube.
7. Once blood starts to flow, the rat head should be downward with the low part of the body up so that capillary tube is facing downwards and blood can be allowed to drip gravitationally. About 2 mL of blood at a time for a series bleeding for every two weeks is possible.
8. After bleeding, capillary tube is removed, the eyelid used for bleeding is closed quickly, and some pressure on the eyeball is put with Kimwipes paper for 30 s.
9. After that, bleeding usually stops immediately. The rat is released gently. Check for a while if bleeding continues.
10. Blood samples stand 1 h at 2-8 C.
11. Serum was separated by centrifugation at 1000 x g ($xG = 1.119 \times 10^{-5} \times rpm^2 \times r[7.3cm]$) for 60 min and stored at –70 C until analysis.

Timing

5 minutes for a rat.

Troubleshooting

Watch carefully not to over-anesthetize the rat.

References

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