A refined protocol combining anesthesia and analgesia within the framework of rabies intracerebral mouse inoculation

Aiello R[1], Canali D[1], De Benedictis GM[2,3], Mutinelli F[4], De Benedictis P[1], Boldrin M[3,4]

[1] FAO and National Reference Centre for Rabies, OIE Collaborating Centre and National Reference Centre for Diseases at the Animal-Human Interface, Istituto Sperimentale delle Venezie, Legnaro, Italy; [2] Department of Animal Medicine, Productions and Health, University of Padua, Legnaro, Italy; [3] CORIT-Consortium for Research in Organ Transplantation, Padua, Italy; [4] Animal Protection Body, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Contact person: raiello@izsvenezie.it

Introduction

Although progressive replacement of rabies intracerebral mouse inoculation has been applied (1) using *in vitro* models (Fig. 1), this technique is still inevitably adopted for the production of reference materials (2). Prevalent anaesthetic protocols do not guarantee proper analgesia and are responsible for side effects, ultimately leading to the animal's death. We aimed at refining a mouse anaesthetic protocol (Fig. 2) to be safely used in young mice by guaranteeing appropriate analgesia and a rapid recovery time.

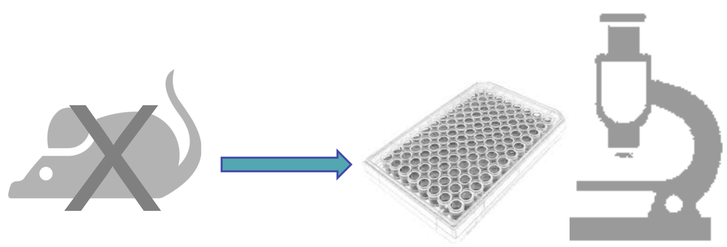


Figure 1. Replacement

Materials and Methods

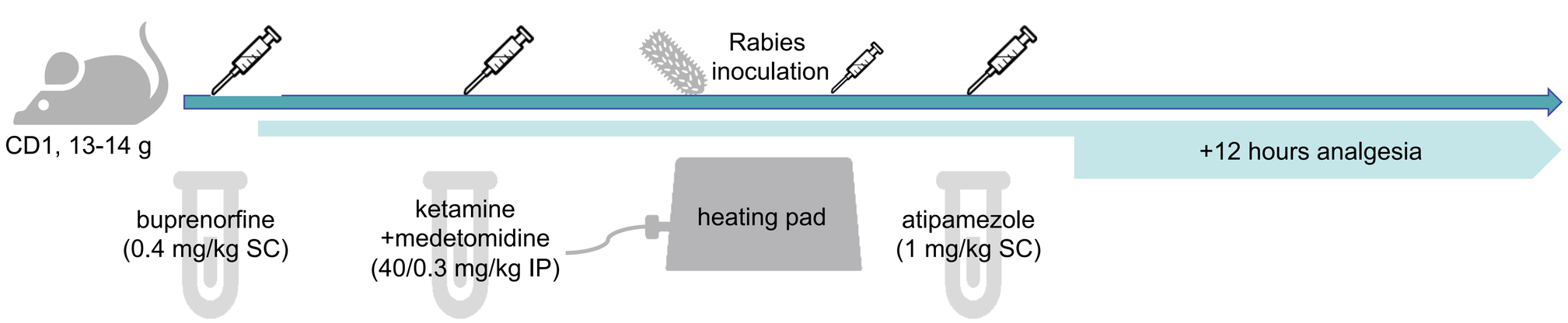


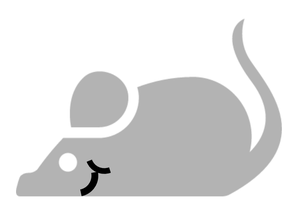
Figure 2. Refined procedure proposed for the rabies intracranial mouse inoculation.

- 178 CD1 3-week-old mice (13-14 g) of both sexes were obtained from an internal breeding facility in four different sessions.
- According to the available literature (3), the tested anesthetic protocol included the administration of 0.4 mg/kg buprenorfine by subcutaneous (SC) route.
- Thirty minutes later, ketamine+medetomidine 40/0.3 mg/kg were administered by the intraperitoneal (IP) route, and animals were placed on a warming pad (+37°C) immediately after the loss of righting reflex.
- After 5 minutes, the absence of nociceptive withdrawal reflex was confirmed and mice were inoculated intracerebrally with 30 µl of virus suspension by a 30 G needle inserted 2 mm under the skull. Animals were then placed again on the warming pad.
- After a15-minute-interval, mice were injected by SC with atipamezole 1 mg/kg as reversal and recovered within few minutes.

Housing and procedures were in accordance with Directive 2010/63/EU and approved by the IZSVe's Ethics Committee.

Results

Anesthetic depth was considered satisfactory in both females and males +
Reversal was obtained after a few minutes and all animals recovered from anesthesia = Refinement



No death was registered within 24 hours = Reduction

Discussion and conclusions

Despite the effective application of *in vitro* techniques for both virus isolation and vaccine potency testing, the complete replacement of the intracerebral mouse inoculation in the practice has still to be achieved.

Although moderate to severe pain due to rabies clinical signs cannot be avoided since their onset represents the experimental endpoint, the severe discomfort due to the procedure itself should be avoided. Inhalation anaesthesia or common injectable anaesthetic protocols are mainly used for mice immobilisation during mild procedures. However, the lack of analgesia makes them unfit for intracerebral inoculation. In addition, they may result impractical due to the long-lasting effect and the related hypothermia and hypoglycaemia.

The proposed protocol is a safe and balanced alternative when a deep anaesthetic plane, rapid recovery and alleviation of post-operatory pain are requested in young laboratory mice. Indeed, the possibility to antagonise medetomidine and the rapid metabolism of ketamine allowed a fast recovery from anaesthesia in all animals, eventually preventing mortality. Considering the unavoidability of such a procedure, this protocol could largely alleviate discomfort for mice used in rabies laboratories.

References

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