Short Communications

AN INNOVATIVE ASSEMBLY TO MAINTAIN ISOFLURANE ANAESTHESIA FOR DENTAL PROCEDURE IN RATS

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SUMMARY

An innovative assembly to maintain isoflurane anaesthesia in rats is described. This assembly was used successfully in laboratory rats for dental procedures that may last as long as sixty minutes. Repeated exposures to isoflurane anaesthesia, of up to eight times within a 30-day study period in healthy laboratory rats did not result in any observable adverse-effects.

Keywords: Isoflurane anaesthesia, anaesthetic circuit, nose cone, rat

INTRODUCTION

Small mammals suchas hamsters, gerbils, guinea pigs, fancy mice and fancy rats are gaining popularity as pets. They may be presented to veterinarians for examination and procedures that may require anaesthesia. The main three methods to anaesthetise these little mammals include the use of injectable anaesthetics alone, inhalant anaesthetic alone, or the combination of injectable and inhalant anaesthetics (Heard, 2004).

The most common anaesthetic agents used for induction and maintenance of anaesthesia in rodents are the combination of ketamine and xylazine. Ketamine is a dissociativeanaesthetic with analgesic properties. Used on its own, ketamine typically results in anaesthesia that is characterised by muscle rigidity and presence of reflexes. Therefore, ketamine is usually combined with another agent that has better muscle relaxation properties. Xylazine is a sedative with good analgesic and muscle relaxation properties; butit may cause significant cardiorespiratory depressions (Albrecht *et al.*, 2014). Therefore, it may be safer and more convenient to maintain the anaesthesia with inhalant anaesthetic rather than re-dosing the rodents using injectable agents (Gargiulo *et al.*, 2012).

Delivery of an inhalant anaesthetic with high vapour pressure such as isoflurane requires the use of a vaporiser to precisely control the split ratios, and delivera safe concentration. In addition, a rodent anaesthetic circuit and nose cone is required. Commercially available rodent circuit is essentially a smaller than standard nonrebreathing circuit, and a small nose cone with replaceable rubber diaphragm. Where these are not immediately available, a simple modification to the commonly used modified Jackson-Rees (MJR) anaesthetic circuit could be a useful alternative. This paper reports the use an innovative adaptation of commonly available items in most veterinary practice to maintain isoflurane anaesthesia safely in laboratory rats for dental procedures.

MATERIAL AND METHODS

In a study to induce periodontal disease in laboratory rats, high doses of ketamine-xylazine, even at 100 mg/kg ketamine and 5 mg/kg xylazine could not abolish chewing reflex satisfactorily to allow dental procedures. Several failed attempts prompted the trial use of MJR-circuit to deliver isoflurane. The patient-end of the MJR circuit has a 22 mm diameter X 20 mm small space; and could fit the rat's snout. It may besuitable to be adapted as a rodent nose cone. Initially, a cut-fingertip of a rubber glove was tried as the diaphragm. Deep anaesthesia could be maintained, however, the rubber glove was too soft, and the rat's snout often got displaced during dental manipulation. In place of the rubber glove, strips of elastic bandage (3M CobanTM, Natural rubber, 15 82 DH-9999-78 Germany) were tried and found to be more suitable. Finally, a plastic frame was used to secure a 15 mm / 22 mm elbow connector. The 15 mm end was connected to the MJR circuit, while the 22 mm end was covered with the elastic bandage to act as the rat's nose cone (Figure 1).

RESULTS AND DISCUSSION

Using this modification, rats can be maintained for dental procedures that may last as long as 60 minutes. This innovative assembly has been used to maintain isoflurane anaesthesia successfully in the study. Anaesthesia was induced by placing the rats into a 10-litre plastic container. A 15 mm / 22 mm connector was fitted onto the top lid of this container to facilitate connection to the MJR circuit. Anaesthesia was typically delivered at 2 L/min oxygen, with 5% isoflurane setting; and rats became anaesthetised within 2 to 3minutes. Rats were then maintained using the assembly for dental procedures.

In the study mentioned above, anaesthesia was required every 3 to 4 days to examine the teeth and oral cavity. In total, the rats were anaesthetised 8 times throughout the 30-day study period. The first anaesthesia sessions were usually the longest, ranging from 15 to 60 minutes. Subsequent sessions ranged 10 to 15 minutes. Following isoflurane anaesthesia, all rats recovered well and resumed their usual activities in the afternoon. The potential toxic effect of repeated anaesthesia was evaluated at the end of the 30-day study period. Under isoflurane anaesthesia, blood was collected via cardiac

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Figure 1. Assembly to deliver inhalant anaesthesia to rat

N, 22 mm end of an elbow connector wrapped with CobanTM to fit the rat's snout; part of CobanTM is anchored to the rat's incisors.

H, the elbow connector is fit through a hole in a plastic frame;

C, 15 mm end of the connector is connected the MJR circuit;

P, plastic frame to secure the connector and accommodate the rat in dorsal position

puncture, followed by euthanasia. Serum were harvested the same day and stored at -20°C until analysis. After euthanasia, the viscera organs were examined grossly.

In this report, the serum creatinineand alanine aminotransferase (ALT) levels for two groups of rats (n=6 per group) are presented (Table 1). Group LP were rats that had 4/0 nylon ligatures placed on both second upper molars and intragingival injection of *Porphyromonas gingivalis* lipopolysaccharide to induce periodontal disease. Group C were rats with no intervention, except for the 8 times of anaesthesia for teeth and oral examination. Both the serum creatinine and ALT levelswere not different between groups, and the values were within the published normal limits (Table 1). The normal limits for creatinine and ALT has been reported as $44.2 - 53.0 \mu mol/L$ and 28.0 - 40.0 U/L, respectively (Giknis and Clifford, 2006).

Table 1. Serum	creatinine	(CREAT)	and alanine
aminotransferase	(ALT)	following	repeated
anaesthesia in rats		0	-

Group	CREAT (µmol/L)	ALT (U/L)
LP	41.16±2.92	35.16±3.97
С	41.5±2.42	35±6.75

Data expressed as mean \pm SD. Group LP, induced periodontal disease. Group C, control. n=6 per group. There were no difference between groups (T-test, α =0.05).

Gross examination of theliver, kidney and spleen in both groups revealed no significant findings. One rat in Group LP showed four pin points of petechiation at the fundus. The finding of gastric ulcer in this rat may be due to stress following induction of periodontal disease.

CONCLUSIONS

In conclusion, a simple assembly, modified from readily available items can be used to deliver inhalation anaesthesia in rats. Practitioners whom possess a small animal anaesthetic machine and MJR circuit may consider this adaptation for the rodent patients. Moreover, results from this study showed that repeated exposures to isoflurane anaesthesia did not cause any observable adverse-effects in the rats.

CONFLICT OF INTEREST

No conflict of interest between the authors.

REFERANCES

- Albrecht, M., Henke, J., Tacke, S., Markert, M., Guth, B. (2014). Influence of repeated anaesthesia on physiological parameters in male Wistar rats: a telemetric study about isoflurane, ketaminexylazine and a combination of medetomidine, midazolam and fentanyl. BMC Veterinary Research. 10: 310.
- Heard, D.J. (2004). Anesthesia, analgesia and sedation of small mammals. In:Ferrets, Rabbits, and Rodent Clinical Medicine and Surgery. Quesenberry, K.E. and Carpenter, J.W. (Eds.) 2nd. Ed., Saunders, St. Louis. pp.356-369.
- Gargiulo, S., Greco, A., Gramanzini, M., Esposito, S., Affuso, A., Brunetti, A., Vesce, G. (2012).Mice anesthesia, analgesia and care. Part I: anaesthetic consideration in preclinical research. ILAR J. Vol: 53(1).E55-69.
- Giknis,M.L.A., Clifford, C.B., (2006).Clinical laboratory parameters for Crl:CD(SD) Rats. Charles River Laboratories.