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Case Report

ALTERNATIVE ANAESTHETIC MANAGEMENT OF RATS FOR MICROSURGERY TRAINING

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In Malaysia, pentobarbital has commonly been used to anaesthetise laboratory animals for teaching and research purposes. In rats, a dose rate of 40–60 mg/kg intraperitoneally could produce anaesthesia lasting for 80-95 minutes (Thurmon *et al.*, 1996). However, pentobarbital at dosages high enough to enable surgery typically would result in severe respiratory depression (Flecknell, 1996). Furthermore, the inability to obtain intravenous access to carefully titrate the required dose for the individual rat necessitates intraperitoneal (IP) bolus administration of pentobarbital, leading to a high mortality rate. Therefore, its use in rats is not recommended. Previous unsatisfactory experience with pentobarbital in a local institution prompted the trial of ketamine-xylazine as an alternative anaesthetic protocol. Ketamine-xylazine has a higher safety margin and causes less cardiovascular depression than pentobarbital (Saha *et al.*, 2007). This case report aims to report the experience of using a prefixed dose of ketamine-xylazine to manage mass anaesthesia for a basic microsurgery workshop.

The workshop involved training on end-to-end anastomosis of arteries. It required the rats to be in a surgical plane for exposure of the carotid or femoral arteries, and to be kept alive for 2 – 2.5 hours to assess the patency of the anastomosed vessel at the end of the exercise. From the literature, a combination of ketamine at 75-100 mg/kg and xylazine at 10 mg/kg, IP, is expected to provide surgical anaesthesia lasting for 20-30 minutes and sleep time of 120-240 minutes (Flecknell, 1996). Therefore, a preliminary trial of using approximately 66.7 mg/kg ketamine and 6.7 mg/kg xylazine, intramuscularly (IM) was performed on two rats, each weighing 385 g and 460 g respectively. Rats were immobilised in 5 minutes. When the rats showed responses to surgical stimuli, tail or toe pinch tests, or return of brisk palpebral reflex, ketamine-xylazine at half of the above induction dose was administered IM. This initial trial showed that this protocol can be used efficiently and safely to maintain anaesthesia for at least 2.5 hours.

On the day of the workshop, it was impossible to administer the exact dose according to the exact body weight of each individual rat, as all the 19 rats needed to be restrained and anaesthetised at once by limited personnel. Thus, we chose to use a fixed anaesthetic volume regardless of the individual body weight. All 19 rats were weighed together and an average weight per rat was calculated. The anaesthetic mixture was prepared by mixing ketamine 100 mg/ml (Ketamil, Troy Laboratories Pty Limited, Australia) with xylazine 20 mg/ml (Xylazil-20,Troy Laboratories Pty Limited, Australia) at a volume ratio of 2:1 into a sterile vial. Consequently, each mL of the mixture contained 66.7 mg/ml ketamine and 6.7 mg xylazine. Given at 0.1 mL per 100g body weight, this mixture would provide 66.7 mg/kg ketamine and 6.7 mg/kg xylazine. Based on the calculated average weight of 220g, 0.22 ml of the mixture was used to induce each of the 19 rats..

All 19 rats were successfully induced and distributed to the trainees within 10 minutes. The trainees had been instructed to instill the surgical site with 1% lignocaine (maximum of 0.5 ml) following surgical exposure. Four personnel monitored the rats for signs of light anaesthesia and responses to surgical stimuli, such as an obvious increase in the depth and rate of breathing, brisk palpebral reflex and movement of jaw, limbs or tail. When top-up was needed, a fixed dose of 0.11 ml of the ketamine-xylazine mixture was given and the time recorded. At the end of the exercise, the rats were euthanised with an overdose of pentobarbital. The time intervals between each top-up for each rat were extracted from the records to calculate the duration of effective anaesthetic action.

The time interval between each top-up of ketamine-xylazine and total anaesthesia time for each rat is presented in Table 1. As two records were missing, the results are based on the remaining 17 records. The interval between induction and the first top-up ranged from 15 to 120 minutes, with a mean ±SD of 43 ±23 minutes, n = 17. If the lower and upper extremes are removed, this would result in 39 ±11 minutes, n = 15.

Following the 1st top-up, three rats died of hypovolemic shock due to surgical bleeding (Rat 12, 13 & 16), while one was euthanised (Rat 5, end of surgical exercise). The remaining 13 rats required a second top-up of ketamine-xylazine after the time interval of 38 ±9 (range 25 – 56) minutes. Following the 2nd top-up, 1 died of sur-

Table 1: Time intervals between induction (In) and subsequent top-ups of ketamine-xylazine, and total anaesthesia time in 17 rats used in microsurgery training

	Time intervals (minutes)				Total anaesthesia
Rat No.	In - 1st Top	1st – 2nd Top/E	2nd- 3rd Top/E	3rd – E	time (minutes)
1	68	25	26; E		119
2	30	30	43	47; E	150
3	39	34	34; E		107
4	29	39	70; E		138
5	120	13;E			133
6	45	35	46; E		126
7	33	42	68; E		143
8	49	41	H		90
9	33	48	? ; E		?
10	25	40	40	37; E	142
11	42	30	44; E		116
12	36	H			36
13	41	H			41
14	30	56	44; E		130
15	50	33	31	30; E	144
16	38	H			38
17	15	50	60; E		125

E – euthanasia with pentobarbitone overdose H- died of haemorrhage ?-time not recorded

gical bleeding (Rat 8), while 9 rats were euthanised. The 2nd top-up lasted for at least 49 ±16 (range 26 – 70) minutes before euthanasia. Time of euthanasia for Rat 9 was not recorded and thus, was not included in the calculation. The remaining 3 rats required a 3rd top- up after the time interval of 38 ±6 (range 31- 43) minutes. In these 3 rats, the 3rd top-up lasted at least 38 ±9 (range 30 – 47) minutes before they were euthanised. The total anaesthesia time was calculated as the time interval from induction to euthanasia or death from surgical bleeding. If the anaesthesia time of the 4 rats that died of surgical bleeding is excluded, the total anaesthesia time is131 ± 13 (range 107-150) minutes, n=12.

There were obvious individual variations in the time interval between induction and subsequent top-ups. This was expected as the total volume of anaesthetics was fixed while rat sizes varied. Nevertheless, the intervals from induction to the 1st top- up at 39 ± 11 minutes (n = 15); 1st to 2nd top-up at 38 ±9 minutes, (n = 13); and 2nd to 3rd top-up at 38 6, (n = 3) appeared consistent. This experience demonstrates that the chosen anaesthetic protocol can be used to efficiently manage anaesthesia of multiple rats and keep them alive for at least 107 minutes. In summary, it is feasible to pre-mix the ketamine-xylazine at 2:1 ratio, and dose at 0.1 ml/100g average body weight for induction and top-up at 0.05 ml/100g to manage anaesthesia of multiple rats simultaneously.

REFERENCES

Flecknell, P.A. (1996). Laboratory Animal Anaesthesia. 2nd ed. Amsterdam. Elsevier Butterworth Heinemann. pp 161-168.

Saha, D.C., Saha, A.C., Malik, G., Astiz, M.E. and Rackow, E.C. (2007). Comparison of cardiovascular effects of tiletamine-zolazepam, pentobarbital, and ketamine-xylazine in male rats. *J. Am. Assoc. Lab. Anim. Sc.* **46**(2): 74-80.

Thurmon, J.C., Tranquilli, W.J. and Benson, G. J. (1996). Lumb & Jones' Veterinary Anesthesia. 3rd ed. Baltimore. Williams & Wilkins. 716p.