Polish Journal of Veterinary Sciences Vol. 23, No. 1 (2020), 127–132

DOI 10.24425/pjvs.2020.132756

Original article

Efficacy comparison of intraperitoneal anaesthesia and post-operative analgesia regimens in laboratory rats

F. Moreno-Mateo[4], B. García-Medrano[1], A. Álvarez-Barcia[2], M.J. Gayoso[3], M.A. Martín-Ferrero[1]

[1] Servicio de Cirugía Ortopédica y Traumatología. Hospital Clínico Universitario de Valladolid, Spain
[2] Bienestar y Salud Animal. Universidad de Valladolid, Spain
[3] Departamento de Biología Celular, Histología y Farmacología. Universidad de Valladolid, Spain
[4] Servicio de Cirugía Ortopédica y Traumatología. Hospital Clínico Universitario de Valladolid, Spain

Abstract

Introduction: Effective and safe anesthesia for rodents has long been a leading concern among biomedical researchers. Intraperitoneal injection constitutes an alternative to inhalant anesthesia.

Purpose: The aim of this study was to identify a safe, reliable, and effective anesthesia and postoperative analgesia protocol for laboratory rats exposed to painful procedures.

Material and methods: Twenty-seven female Wistar rats in an ongoing study that required surgery were randomized into groups for three different intraperitoneal anesthesia protocols and three different analgesia regimens. The anesthesia groups were (1) medetomidine + ketamine (MK), (2) ketamine + xylacine (KX), and (3) fentanyl + medetomidine (FM). Three analgesia groups were equally distributed among the anesthesia groups: (1) local mepivacaine + oral ibuprofen (MI), (2) oral tramadol + oral ibuprofen (TI), and (3) local tramadol + oral tramadol + + oral ibuprofen (TTI). A core was assigned to measure anesthesia (0-3) and analgesia (0-2) effectiveness; the lower the score, the more effective the treatment.

Results: The mean MK score was 0.44 versus 2.00 for FM and 2.33 for KX. Mean score for analgesia on the first postoperative day was TTI (4.66) TI (9.13), and MI (10.14). Mean score 48 hours after surgery was TTI (3.4), TI (6.71), and MI (9.5). These differences were statistically significant.

Conclusion: MK was shown to be a reliable, safe, and effective method of anesthesia. The TTI analgesia regimen is strongly recommended in light of these results.

Key words: pain in rats, anesthesia for rodents, analgesia for rodents, laboratory rats, animal welfare

Correspondence to: F. Moreno-Mateo, e-mail: fmorenomateo@gmail.com

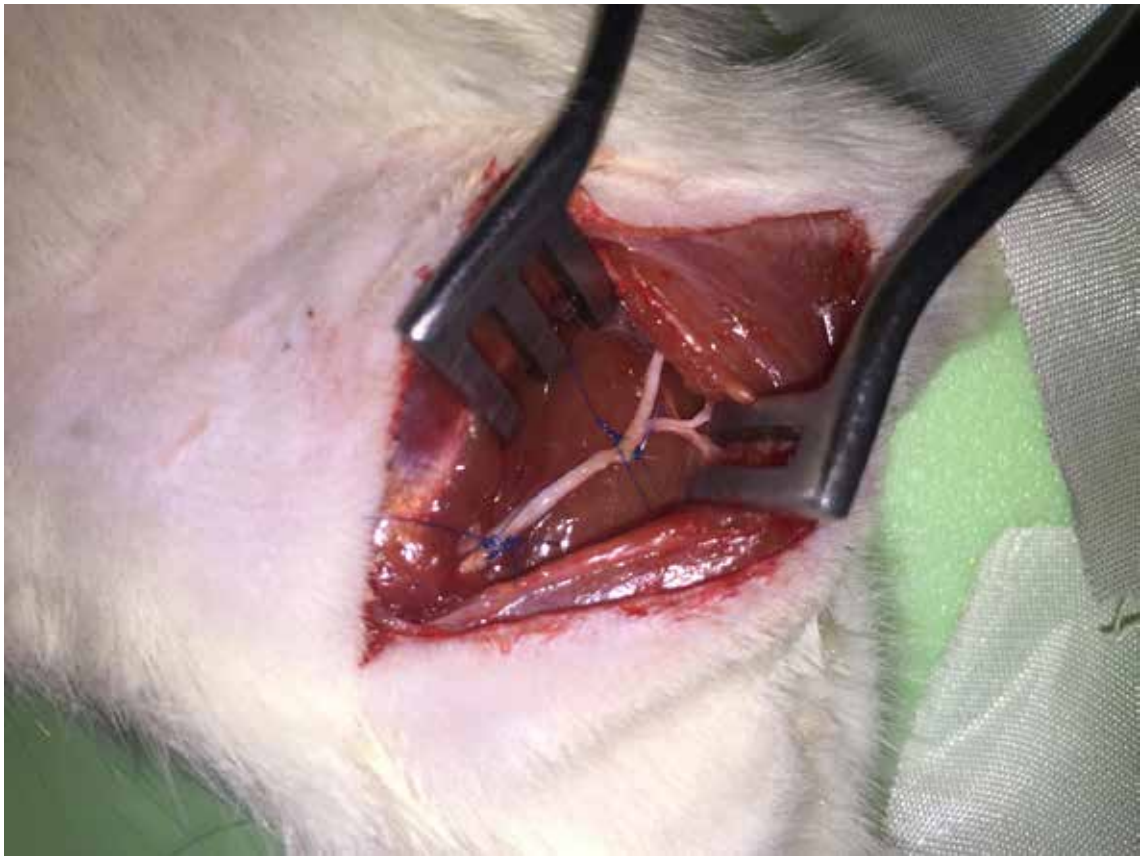

Fig. 5. Surgical procedure showing the right sciatic nerve allograft implanted through the longitudinal posterolateral approach.

Introduction

Rodents are widely used in many types of surgical research and academic procedures. Interest in animal welfare has been increasing in recent decades due to regulatory and ethical considerations (Cardozo et al. 2007, Romero et al. 2016). Recent studies have also shown better results and clinical outcomes when appropriate therapies had been applied to prevent animal pain and stress (Kohn 2007). Effective and safe anesthesia for rodents has long been a leading concern among biomedical researchers. Inhalant anesthesia is known to be a safe and effective technique that also allows rapid recoveries so that hypothermia and hypoxia are prevented. However, the necessary specific delivery equipment is not always available, and his equipment has some experimental limitations. Intraperitoneal injectable anesthetic protocols are used in rodents to avoid these limitations.

In a similar way, many different protocols have been described to treat and prevent post-surgical pain in rats (Claire et al. 2013). In the particular field of orthopedic research, the animals are potentially exposed to the highest levels of pain during the procedures. For this reason it is essential to find a suitable and reproducible protocol for these types of procedures.

The aim of the present study was to assess and compare the levels of anesthesia and postoperative pain in rats administered with three different anesthesia and analgesia protocols.

The null hypothesis stated that there was no difference between groups.

Materials and Methods

Animals and facilities

To adhere to the principle of reduction, the studied animals were selected in the setting of another clinical trial on sciatic nerve critic defect and its ulterior reparation by two different methods. The right sciatic nerve was exposed between the vastus lateralis and the biceps femoris using the longitudinal posterolateral approach. The nerve was then dissected from the surrounding tissue with a surgical microscope, and a 1 cm portion was resected using microsurgical instruments. The nerve defect was then reconstructed using a 1cm allograft in the control group and a 1cm xenograft processed using new decellularization method in the study group (Fig. 5). In terms of pain, both methods were considered to be equal. Female Wistar rats were used (n = 27, weight 214-292 g, age 8-12 weeks). They were housed

Table 1. Drug dosages.

Drug	Dosage
Ibuprofen PO	100 mg dissolved in 0.5 l of H_2O (free access)
Tramadol PO	5 mg/kg/day
Tramadol IM	2.5 mg/kg
Mepivacaine 1%	0.1 mL
Ketamine + xylacine	80 mg/kg + 10 mg/kg
Fentanyl + medetomidine	0.3 mg/kg + 0.3 mg/kg
Medetomidine + ketamine	0.3 mg/kg + 80 mg/kg

Table 2. Post-surgical pain score.

Behaviour	Normal	0
	Less motion than usual Animal avoids being touched	0.1
	Still, totally motionless Aggressive	0.4
Porphyrins	None	0
	Mild (eyes or nose)	0.1
	Obvious (face or pads)	0.4
Gait and position	Normal	0
	Mild incoordination when stimulated, mild piloerection	0.1
	Ataxia or head tremor	0.4
Weight loss	0%-5% (1-3 post operative day)	0
	5%-10%	0.1
	>10%	0.4
Appetite	Normal	0
	Rat does not eat dry food, (only fruits or chocolate spread). Rat drinks and is hydrated	0.1
	No interest in food. Signs of dehydration	0.4
Total		

in an accredited rodent facility with a 12:12 light-dark cycle in a room with controlled temperature (21°C) and humidity (55%).

The animals were randomized into nine groups, as follows:

A: Anesthesia: medetomidine + ketamine
Analgesia: mepivacaine + ibuprofen

B: Anesthesia: medetomidine + ketamine
Analgesia: tramadol (by mouth, PO) + ibuprofen PO

C: Anesthesia: medetomidine + ketamine
Analgesia: tramadol (intramuscularly, IM) + tramadol PO + ibuprofen PO

D: Anesthesia: fentanyl + medetomidine
Analgesia: tramadol PO + ibuprofen (PO)

E: Anesthesia: fentanyl + medetomidine
Analgesia: mepivacaine + ibuprofen

F: Anesthesia: fentanyl + medetomidine
Analgesia: tramadol IM + tramadol PO + + ibuprofen PO

G: Anesthesia: ketamine + xylacine
Analgesia: tramadol PO + ibuprofen PO

H: Anesthesia: ketamine + xylacine
Analgesia: mepivacaine + ibuprofen

I: Anesthesia: ketamine + xylacine
Analgesia: tramadol IM + tramadol PO + + ibuprofen PO

Thus, there were three anesthesia groups (MK, FM, and KX) and three analgesia groups (TI, MI, and TTI). The drug dosages are provided in Table 1.

Drug administration and procedure

Oral ibuprofen was administered dissolved in water, whereas oral tramadol was administered mixed in a chocolate spread (Nocilla®) (Goldkuhl 2008). The rats had access to the chocolate spread without tramadol the day before surgery to allow them to become familiar with it. Parenteral tramadol was

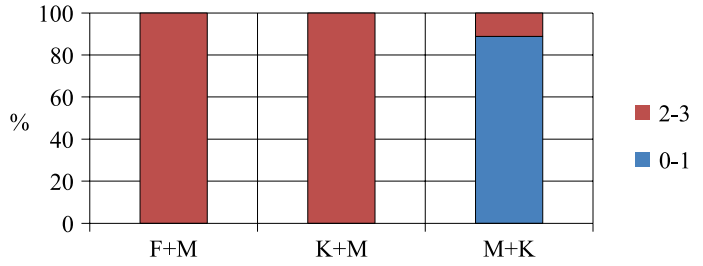

Fig. 1. Statistically significant notable differences were observed when signs of pain during surgery were noted (Chi-Square). All the rats in the FM and KM groups scored 2 or 3 points, while most of the MK group individuals scored 0 or 1 point.

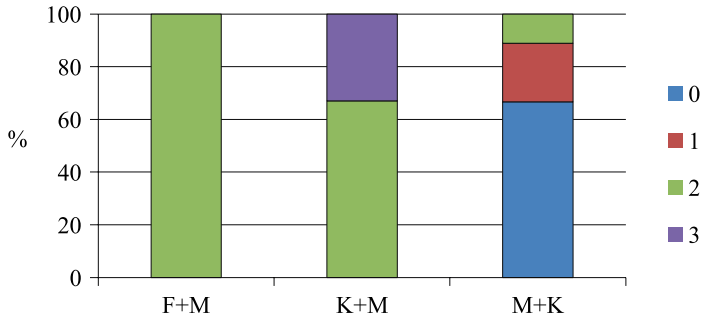

Fig. 2. Statistically significant notable differences were observed when signs of pain during surgery were noted (Chi-Square). All the rats in the FM and KM groups scored 2 or 3 points, while most of the MK group individuals scored 0 or 1 point.

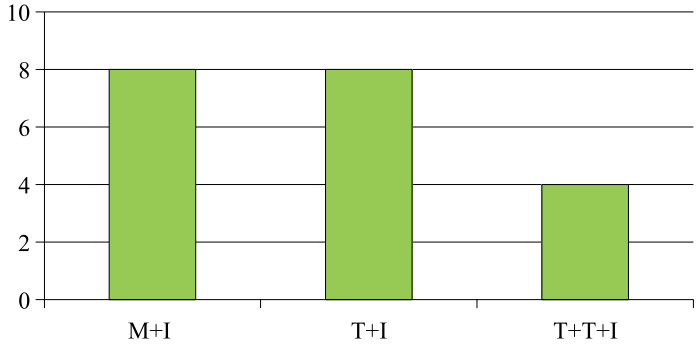

Fig. 3. 1st post-op day pain score. Median. Kruskal Wallis.
TTI group showed the best result in terms of pain control during the first and second postoperative days.

administered on the surgical site, just over the treated nerve.

The intraperitoneal injection was administered after 5 to 10 minutes handling the animals to get them relaxed (Mourelle et al 2013). In this way, the animals did not need to be immobilized, avoiding the stress this causes them. The anesthetic mixture was then injected using a 23G × 25 mm needle at a 45° angle to the abdominal wall in the lower right quadrant. The rats usually remained calm during the procedure and did not show any sign of pain. On seven occasions, the rats showed signs of pain during the intraperitoneal injection and moved defensively; all of these animals required a second dose (Waynforth et al. 1992, Jaber et al. 2014). During the surgical procedure, vital signs were monitored every 5 minutes and at the moment of maximum expected pain. An electric blanket was used during surgery and recovery until the animal started to move. The anesthetic parameters measured were as follows (Dannenman et al. 1997, Alves et al. 2010): induction time, loss of pedal reflex, signs of pain during surgery (e.g., withdrawal reflex, tachycardia/ /tachypnea, sounds emitted), and recovery time. Points were assigned as follows: 0 points if no intraoperatory events were noted; 1 point if only a withdrawal reflex was noted; 2 points if tachycardia or tachypnea were registered; and 3 points if any sound was emitted during the procedure. In the few cases in which a sound was emitted, it was during the nerve section.

Analysis of variance (ANOVA) was used to analyze induction time, and the chi-square test was used for intraoperatory signs and number of exitus related to the different anesthetic protocols.

The analgesic parameters measured during postoperative days 1, 2, and 5 were as follows: respiratory rate, height, feeding, feces, chromodacriorrea, move-

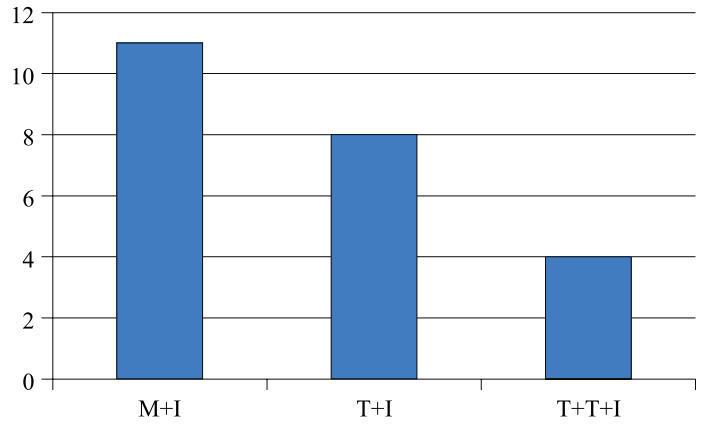

Fig. 4. 2nd post-op day pain score. Median.

ment, grooming, position, and response to stimuli. A numeric scale following The University of Zaragoza recommendation was used to measure postoperative pain according (Table 2). The three groups were compared and contrasted using the Kruskal-Wallis test.

All of the experiments were performed in a homologated laboratory according to European Union and Spanish Government guidelines for the ethical care of animals (EU Directive 63/2010, RD 53/2013), and the study was approved by the ethical commission for animal research at our institution.

Results

The longest and shortest induction times were observed in the KX and KM groups, respectively, but the difference was not significant. The shortest recovery time was in the FM group, followied by KM; the longest was in MK, lasting in some cases more than 6 hours. Statistically significant notable differences were observed when signs of pain during surgery were studied, with the best score in the pain scale in the MK group (0.44), followed by FM (2) and KX (2.33) (Figs. 1 and 2).

Regarding the pain score punctuation after surgery, no significant differences were observed on postoperative day 5. On postoperative day 1, the best analgesic protocol was TTI (4.66), whereas the TI (9.13) and MI (10.14) groups were very similar (Fig. 3). The main significant differences between the three analgesic protocols were found on postoperative day 2 TTI (3.4), TI (6.71), and MI (9.5), ($p<0.05$) (Fig. 4).

Five exitus were noted (two in the KX+TI group, one in the FM+MI group, and two in the FM+MI group). These differences were not statistically significant.

Discussion

Increased adverse effects have been reported for the MK protocol (Wellington et al. 2013). In our study, however, despite administering medetomidine near its upper security limit, we did not notice any adverse effect besides its long recovery period. A possible explanation could be the lower dose of ketamine used in our experiment. Medetomidine has also shown a protective effect against cerebral ischemia (Hoffman et al 1991). The long recovery period can be managed with appropriate postoperative care, including respiratory, heart rate, and temperature monitoring. The use of an electric blanket and providing a stable environment are recommended. We did not use any antidote, but MK anesthesia could also be reversed by atipamezole (Hedenqvist et al 200 and Jang et al 2009). The unexpected positive result in pain reduction of tramadol administered on the surgical site could be explained by the peripheral effects of this drug (Hancı et al. 2012, Sousa et al. 2014) added to a well-known central nervous system pain modulation.

The sum of our clinical observation suggests that the MK anesthetic protocol is a reliable, safe, and effective method. The TTI analgesia regimen is strongly recommended in the light of these results. Both regimens have been shown to be safe, with a low exitus rate.

Acknowledgements

Project funded by Fundación SECOT.

References

ACLAM Task Force Members, Kohn DF, Martin TE, Foley PL, Morris TH, Swindle MM, Vogler GA, Wixson SK (**2007**) Public statement: guidelines for the assessment and management of pain in rodents and rabbits. J Am Assoc Lab Anim Sci 46: 97-108.

Alves HN, da Silva AL, Olsson IA, Orden JM, Antunes LM (**2010**) Anesthesia with intraperitoneal propofol, medetomidine, and fentanyl in rats. J Am Assoc Lab Anim Sci. 49: 454-459.

Danneman PJ, Mandrell TD (**1997**) Evaluation of five agents/ /methods for anesthesia of neonatal rats. Lab Anim Sci 47: 386-395.

Hankenson FC (**2013**) Critical Care Management for Laboratory Mice and Rats. 1st. ed., CRC Press, Taylor and Francis Group.

Goldkuhl R, Carlsson HE, Hau J, Abelson KS (**2008**) Effect of subcutaneous injection and oral voluntary ingestion of buprenorphine on post-operative serum corticosterone levels in male rats. Eur Surg Res 41: 272-278.

Hancı V, Hakimoğlu S, Özaçmak H, Bektaş S, Özaçmak HS, Özdamar SO, Yurtlu S, Turan IÖ (**2012**) Comparison of the effects of bupivacaine, lidocaine, and tramadol infiltration on wound healing in rats. Rev Bras Anestesiol. 62: 799-810.

Hedenqvist P, Roughan JV, Flecknell PA (**2000**) Sufentanil and medetomidine anaesthesia in the rat and its reversal with atipamezole and butorphanol. Lab Anim. 34: 244-251.

Hoffman WE, Kochs E, Werner C, Thomas C, Albrecht RF (**1991**) Dexmedetomidine improves neurologic outcome from incomplete ischemia in the rat. Reversal by the alpha 2-adrenergic antagonist atipamezole. Anesthesiology. 75: 328-332.

Jaber SM, Hankenson FC, Heng K, McKinstry-Wu A, Kelz MB, Marx JO (**2014**) Dose regimens, variability, and complications associated with using repeat-bolus dosing to extend a surgical plane of anesthesia in laboratory mice. J Am Assoc Lab Anim Sci 53: 684-691.

Jang HS, Choi HS, Lee SH, Jang KH, Lee MG (**2009**) Evaluation of the anaesthetic effects of medetomidine and ketamine in rats and their reversal with atipamezole. Vet Anaesth Analg 36: 319-327.

Mourelle AC, Herrero E, Ricca M (**2013**) Recommendations for manipulation and fastening of laboratory mice and rats. Spei Domus 9: 39-47.

Romero-Fernandez W, Batista-Castro Z, De Lucca M, Ruano A, García-Barceló M, Rivera-Cervantes M, García--Rodríguez J, Sánchez-Mateos S (**2016**) The 1, 2, 3 of laboratory animal experimentation. Rev Peru Med Exp Salud Publica. 33: 288-299.

Sousa AM, Ashmawi HA. Braz (**2015**) Local analgesic effect of tramadol is not mediated by opioid receptors in early postoperative pain in rats. J Anesthesiol. 65: 186-190.

Waynforth HB, Flecknell P (**1992**) Experimental and surgical techniques in the rat, 2nd ed., Academia Press.

Wellington D, Mikaelian I, Singer L (**2013**) Comparison of ketamine-xylazine and ketamine-dexmedetomidine anesthesia and intraperitoneal tolerance in rats. J Am Assoc Lab Anim Sci 52: 481-487.