Evaluation of Xylazine, Acepromazine and Medetomidine with Ketamine for General Anaesthesia in Rabbits

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Summary

A randomized, prospective, blinded experimental study was conducted in 32 rabbits of either sex to compare the anaesthetic and physiological effects of ketamine with different pre-anaesthetics. Rabbits were randomly divided into 4 equal groups. Xylazine 6 mg/kg in animals of group xylazine-ketamine (XK), acepromazine 2 mg/kg in animals of group acepromazine-ketamine (AK), medetomidine 125 µg/kg in group medetomidine-ketamine 1 (MK1) or medetomidine 250 µg/kg in group medetomidine-ketamine 2 (MK2) were administered by intramuscular injection (IM). Five minutes later, ketamine 60 mg/kg was administered intramuscularly to all the groups. The rabbits were observed for the onset of weak time, down time, the time to loss of righting reflex, pedal reflexes and response to surgical stimuli. Heart rate, respiratory rate and rectal temperature and arterial oxygen saturation of haemoglobin (SpO_2) were recorded up to 60 min. Weak time, down time and time to loss of righting reflex were the shortest in animals of group MK2 as compared to the other groups. Pedal reflexes remained intact in all the animals of XK group, but were abolished in 50% of the AK group, 75% of the MK1 group and 100% of animals in the MK2 group. Pain was evinced during surgery by all the animals in group XK, 5 animals in group AK and 4 animals in group MK1. The best analgesia was achieved in the animals of group MK2, where none of the animals showed pain on surgical stimulation. Heart rate and SpO_2 decreased significantly ($P<0.01$) in the animals of groups XK, MK1 and MK2 but respiratory rate and rectal temperature decreased significantly ($P<0.01$) in all the groups. However, all the animals recovered from anaesthesia without complications. It was concluded that medetomidine 250 µg/kg and ketamine 60 mg/kg produced excellent anaesthesia to allow pain free surgery and may be considered suitable for anaesthesia in New Zealand White rabbits.

Introduction

Rabbits are used for experimental studies in different research organisations all over the world and frequently need to be anaesthetized. However, very high peri-anaesthetic mortality (1 in 72) has been reported in rabbits *(Brodbelt et al., 2005)*. The relatively small size and high metabolic rate may be related to high mortality in this species *(Grint and Murison, 2008)*. Furthermore, rabbits are hind gut fermenters and may be prone to gut stasis during and after anaesthesia, if adequate analgesia is not provided *(Stasiak et al., 2003)*.

Many investigators have studied the effects of different anaesthetic drugs in rabbits but there appears to be an increasing popularity of ketamine-based combinations for rabbit anaesthesia, considering the safety and good analgesic properties of the drug *(Henke et al., 2005; Orr et al., 2005; Grint and Murison, 2008)*. However, there seems to be no agreement among the various reports about the use of drugs and their doses to produce reliable anaesthesia in the rabbits. Avsaroglu *et al. (2003)* reported wide variation in response to anaesthesia in different strains of rabbits, whereas Hedenqvist

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Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

et al. *(2002)* and Grint and Murison *(2008)* reported failure to achieve complete anaesthesia with the use of xylazine or medetomidine along with ketamine in rabbits. The rabbits thus sustain lots of suffering and pain due to inadequate analgesia during surgical interventions in experimental and clinical settings. The present study was designed to compare xylazine-ketamine, acepromazine-ketamine and medetomidine-ketamine combinations for general anaesthesia in rabbits.

Materials and Methods

The study was approved by the Animal Ethics Committee of the Institute. Thirty two New Zealand White rabbits of both sexes ranging from 8-10 months of age and 1.6-2.0 kg body weight were included in the study. The animals were housed in individual cages and acclimatized to handling and animal housing conditions for 15 days. During the pre- and post-operative period, feed and water were made available *ad libitum* to all the animals. The animals were prepared for the experiment by clipping and shaving hair at the ventral midline one day before the start of the experiment.

Rabbits were randomly divided into four groups of eight animals (4 animals/sex). The groups were designated as xylazine-ketamine (XK) group, acepromazine-ketamine (AK) group, medetomidine 125 µg-ketamine (MK1) group and medetomidine 250 µg-ketamine (MK2) group. Xylazine 6 mg/kg, acepromazine 2 mg/kg, medetomidine 125 µg/kg and and medetomidine 250 µg/kg were administered in animals of groups XK, AK, MK1 and MK2, respectively, by intramuscular injection. Five minutes later, ketamine 60 mg/kg was administered intramuscularly in the animals of all the groups. The rabbits were placed on a big operation table after the injection of pre-anaesthetic and were observed for the following parameters.

Weak time: The time from the injection of pre-anaesthetic to the time of onset of signs of drowsiness was recorded as weak time.

Down time: This was recorded as the time from the injection of pre-anaesthetic to the time when the animal became recumbent.

Time to loss of righting reflex: Once the animals had become recumbent the recording for the time to loss of righting reflex was initiated. The time from the injection of the pre-anaesthetics to the time when the animal was unable to attain sternal recumbency in response to the digital pressure applied at an interval of one minute to its paw in the forelimb was considered as the time to loss of righting reflex. The time of loss of righting reflex was considered as the time of onset of analgesia

Pedal reflex: Status of the pedal reflex was recoded as intact or abolished at 5 minute intervals up to 60 minutes by applying digital pressure to a hind paw.

Heart rate: Heart rate was recorded with the help of a pulse oxymeter via a probe connected to the ear of the animal before injection of any drug (0 minute), and then at 10, 20, 30, 45 and 60 minutes after the injection of pre-anaesthetic drug.

Respiratory rate: This was measured by counting the thoracic excursion of the animal before injection of any drug (0 minute), and then at 10, 20, 30, 45 and 60 minutes after the injection of pre-anaesthetic drug.

Rectal temperature: This was recorded, using a digital thermometer, before injection of any drug (0 minute), and then at 10, 20, 30, 45 and 60 minutes after the injection of pre-anaesthetics.

Oxygen saturation of haemoglobin: A pulse oximeter was used to measure the arterial oxygen saturation of haemoglobin (SpO_2) via a lingual probe before injection of any drug (0 minute), and then at 10, 20, 30, 45 and 60 minutes after the injection of pre-anaesthetic drug.

Response to surgical stimuli: Ten minutes after the injection of ketamine the animals were subjected to surgery which involved cystotomy through a midline laparotomy approach. If the animal responded to surgical incision or manipulation, the depth of anaesthesia was considered inadequate and infiltration of local anaesthesia was performed at the surgical site in order to complete the surgical procedure.

Statistical Analysis: Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were

used to compare the means between the groups at the various intervals. The values at different time intervals in each group were compared with the respective base values (before drug injection) for different parameters using paired "t" test as per the methods outlined by Snedecor and Cochran *(1994)*. Significance was considered at $P < 0.05$.

Results

Weak time, down time and time to loss of righting reflex were shortest in animals of group MK2 as compared to the other groups (Table 1). Weak time in MK2 group (2.63±0.42) was significantly (P<0.05) shorter as compared to that in the animals of group XK (5.38±1.28), but did not differ significantly (P>0.05) from that in AK and MK1 groups. Down time was significantly (P<0.05) shorter in both MK1 (5.25±0.94) and MK2 (4.75±0.59) groups as compared to the animals of group XK (9.25±2.02). Time to loss of righting reflex was significantly (P<0.05) shorter in MK2 group (7.63±1.03) as compared to the other three groups. Increasing the dose of medetomidine from 125 to 250 µg/kg resulted in faster onset of anaesthesia.

Pedal reflex remained intact in all the animals of XK group, but was abolished for 10 minutes in 4 animals (50%) of AK group and for 10-15 minutes in 6 animals (75%) of MK1 group. In the rabbits

Table 1. Mean ± SE of weak time, down time and time to loss of righting reflex in animals of different groups

Groups	Parameters		
	Weak time	Down time	Righting reflex
XK	5.38±1.28[a]	9.25±2.02[a]	14.00±0.27[a]
AK	4.25±0.31[ab]	7.25±0.53[ab]	12.35±1.40[a]
MK1	3.63±0.57[ab]	5.25±0.94[b]	11.86±1.33[a]
MK2	2.63±0.42[b]	4.75±0.59[b]	7.63±1.03[b]

a, b, c Value with different superscripts different significantly from each other for the particular parameter (P<0.05)

Table 2. Mean ± SE of Heart rate in animals of different groups.

Groups	Time intervals					
	0	10	20	30	45	60
XK	254.50± 11.73	214.63± 13.11	196.38± 11.77*	193.75± 11.62**	185.75± 11.95**	212.50± 12.78*
AK	192.75± 18.78	230.88± 8.51*	218.25± 9.62	203.00± 11.12	201.88± 11.53	186.38± 4.31
MK1	237.25± 11.98	175.25± 10.24**	184.75± 9.55**	192.63± 10.47**	185.88± 6.27**	181.25± 10.90**
MK2	225.25± 11.77	175.88± 9.58*	179.00± 6.41*	157.13± 5.03**	156.75± 6.70**	148.75± 8.44**

Value with asterisks different significantly from the respective base values (* P<0.05; **P<0.01)

Table 3. Mean ± SE of Respiratory rate in animals of different groups.

Groups	Time intervals					
	0	10	20	30	45	60
XK	108.00± 6.54	58.25± 4.56**	54.00± 4.28**	54.50± 4.47**	66.55± 5.34**	69.00± 5.74**
AK	87.50± 12.50	52.00± 7.17**	42.00± 3.70**	43.00± 3.66**	38.25± 3.55**	38.50± 3.79*
MK1	150.63± 9.70	116.00± 6.58**	109.50± 4.45**	99.75± 1.53**	93.00± 2.88**	93.38± 5.78**
MK2	95.63± 4.83	75.00± 6.49*	65.00± 5.57**	52.50± 6.28**	51.63± 3.01**	50.38± 1.67**

Value with asterisks different significantly from the respective base values (* P<0.05; **P<0.01)

of MK2 group, pedal reflex was abolished for 45-60 minutes in all the animals. Pain was evinced by all the animals of group XK at the time of surgical incision and the surgery was performed under local infiltration analgesia. In group AK, 5 animals and in group MK1, 4 animals showed signs of pain on surgical incision, however, the rest of the animals did not show signs of pain and could be operated upon without the use of local anaesthesia. In MK2 group none of the animal evinced pain on surgical stimulation.

Heart rate decreased in the animals of groups XK (P>0.05), MK1 (P<0.01) and MK2 (P<0.05) within 10 minutes after the administration of drugs (Table 2). The bradycardia persisted until the end of the observation period in all the 3 groups though a little improvement in heart rate was recorded in XK group towards the end of the observation period. Respiratory rate also decreased significantly (P<0.01) 10-20 minutes after the administration of drugs in all the groups and remained so until the end of the observation period (Table 3). Similarly, rectal temperature decreased significantly (P<0.05 or P<0.01) in all the groups through out the period of observation (Table 4).

SpO_2 was minimal between 10 and 20 minutes after the administration of the drugs, and tended to increase gradually towards the end of the observation period. In group AK SpO_2 did not change significantly and remained significantly higher than that in other groups. The animals of group XK also had higher values for SpO_2 as compared to that in the animals

Table 4. Mean ± SE of Rectal temperature in animals of different groups.

Groups	Time intervals					
	0	10	20	30	45	60
XK	38.61± 0.21	38.10± 0.14*	37.19± 0.22**	36.51± 0.34**	35.95± 0.31**	35.56± 0.28**
AK	38.41± 0.28	37.79± 0.22*	37.45± 0.28**	37.21± 0.16**	36.90± 0.24**	36.64± 0.30**
MK1	38.60± 0.21	37.58± 0.18**	36.90± 0.16**	36.26± 0.25**	35.80± 0.27**	36.01± 0.24**
MK2	37.29± 0.43	36.50± 0.33*	36.16± 0.28*	35.56± 0.29**	35.44± 0.23**	35.45± 0.20**

Value with asterisks different significantly from the respective base values (* P<0.05; **P<0.01)

Table 5. Mean ± SE of SpO_2 in animals of different groups.

Groups	Time intervals				
	10	20	30	45	60
XK	88.00±0.87	88.63±2.39	92.38±2.20	94.75±1.35**	95.25±1.19**
AK	94.00±2.41	93.88±2.50	93.25±2.45	96.26±1.74	97.75±1.22
MK1	78.88±2.16	83.25±1.74	86.75±2.54**	94.50±1.12**	96.50±0.87**
MK2	78.00±3.11	78.13±2.80	81.00±1.65*	85.88±1.73**	91.38±1.74**

Value with asterisks different significantly from the respective 10 min values (* P<0.05; **P<0.01)

of groups MK1 and MK2. The lowest values for SpO_2 were recorded in the animals of MK2 group until the end of the observation period (Table 5).

Discussion

Ketamine has been commonly used with xylazine for anaesthesia in rabbits. Hedenqvist *et al. (2002)* used xylazine (4 mg/kg) and ketamine 50 mg/kg in rabbits but could achieve surgical anaesthesia only for a short duration in 7 out of 19 rabbits. In our pilot trials we also failed to get adequate depth of anaesthesia with the use of xylazine (5 mg/kg) and ketamine (50 mg/kg) in New Zealand White rabbits. In the present study, therefore, a slightly higher dose of ketamine was selected for use with xylazine, acepromazine and two doses of medetomidine.

Medetomidine is a potent α_2-adrenoceptor agonist that induces sedation, analgesia and muscle relaxation *(Navalainen et al., 1989)*. Its mechanism of action is similar to that of xylazine, which causes sedation and analgesia by stimulating central presynaptic $\alpha 2$ – adrenoceptors, resulting in inhibition of norepinephrine release from adrenergic nerve terminals. Medetomidine has been successfully combined with ketamine for anaesthesia in a range of species including rabbits *(Sakaguchi et al., 1996; Kilic, 2004)*. In rabbits, medetomidine has been reported to produce sedation and some analgesia at doses of 0.25, 0.35 and 0.50 mg/kg IM *(Blum et al., 1992)*. In the present study we used a lower dose of medetomidine to minimize side effects of the drug. Rapid onset of effect in medetomidine groups was recorded in the present study, which conformed to the findings of Kilic *(2004)*, who recorded loss of righting reflex 5 minutes after the administration of 0.25 mg/kg medetomidine and 50 mg/kg ketamine in rabbits. Slightly slower onset of effects in the animals of XK and AK groups is possibly attributable to the slower onset of action of these drugs as compared to medetomidine.

The best analgesia was achieved in group MK2, where none of the animals evinced pain on surgical stimulation and all the animals could be operated upon without the use of local anaesthesia. While evaluating anaesthetic depth, it was noted that sometimes the pedal withdrawal reflex was absent but a positive response was observed when the stimulus was repeated. The first stimulus seemed to arouse the animal to a state in which a repeated stimulus evoked a response *(Kilic, 2004)*. Since most surgical procedures include repetitive nociceptive stimulation, it seems logical to evaluate reflexes by repeating the stimulus when evaluating anaesthetic depth *(Kilic, 2004)*. However, surgical stimulus seems to be an even better indicator of depth of anaesthesia as some of the animals that showed complete loss of pedal reflex on repeated stimulus in the present study showed signs of pain on surgical incision. The depth of anaesthesia was considered excellent in animals of group MK2 as all the animals showed loss of pedal reflexes and absence of pain on surgical stimulation.

A significant decrease in heart rate, as recorded in groups XK, MK1 and MK2, is considered as a classical response following administration of alpha-2 agonists in many species *(DeRossi et al., 2003; Singh et al., 2004)*. Bradycardia following the administration of alpha-2 agonists might be caused by

inhibition of sympathetic tone from CNS, inhibition of norepinephrine release from sympathetic nerve terminals, vagal stimulation due to vasoconstriction and a direct increase in the release of acetylcholine from parasympathetic nerves in the heart *(MacDonald and Virtanen, 1992)*. The bradycardia in these animals could have been even more pronounced, but the positive chronotropic effect of ketamine might have counterbalanced some of the bradycardiac effect of the α_2-agonists *(Kilic, 2004)*. The changes in heart rate in the animals of AK group were non-significant except for a significant increase in heart rate at the 10-minute interval. Acepromazine-ketamine has been reported to cause a significant increase in the heart rate in cats *(Tranquilli et al., 1988)* and dogs *(Farver et al., 1986)*.

Decrease in respiratory rate conforms to the findings of Kilic *(2004)* and Grint and Murison *(2008)*, who reported decreased respiratory rate following the administration of xylazine-ketamine, medetomidine-ketamine and midazolam-ketamine in rabbits. The depression of respiratory function is not unique to the use of these combinations in this species, but also occurs with other injectable anaesthetic regimens *(Borkowski et al., 1990)*. As recorded in the present study, Orr *et al. (2005)* also reported that SpO_2 values in rabbits anaesthetized with medetomidine combinations were often low, which they attributed to peripheral vasoconstriction. In dogs, medetomidine-ketamine anaesthesia significantly decreased arterial oxygen tension *(Ko et al., 2000)*, which can lead to haemoglobin desaturation. This decrease in arterial oxygen tension was thought to be due to a reduction in respiration rate. In our study also respiratory rate was significantly lower in MK1 and MK2 groups as compared to that in XK and AK groups. Orr *et al. (2005)* reported that all anaesthetic techniques commonly used in rabbits depress ventilation. In our study, animals of all groups recovered from anaesthesia after surgery without any complication and no death was recorded in any of the groups, and suggested that cardiovascular depression had little effect on overall welfare of the animals during the study.

The decrease in rectal temperature observed in rabbits after the administration of xylazine, acepromazine or medetomidine with ketamine might have been caused by sedation, reduced metabolism, muscle relaxation and depression of the CNS as suggested by Kinjavdekar *et al. (2000)*. In addition, α_2-agonists may induce prolonged depression of thermoregulation as reported by Ponder and Clarke *(1980)* in cats. All phenothiazine derivatives including acepromazine have also been reported to cause a fall in body temperature *(Hall et al., 2001)*.

It was concluded that the medetomidine-ketamine combination produced better anaesthesia than the combination of xylazine-ketamine or acepromazine-ketamine. This combination produced rapid onset and a reliable and adequate depth of anaesthesia to allow a short duration of abdominal surgery in rabbits without the use of local anaesthetics or administration of a supplemental dose of anaesthetics. Although the combination caused a significant decrease in heart rate, respiration rate and rectal temperature, the changes were only transient and animals recovered from anaesthesia without complications. The combination thus may be considered safe for anaesthesia in New Zealand White rabbits.

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