Iranian Journal of Veterinary Research, University of Shiraz, Vol. 8, No. 2, Ser. No. 19, 2007

Evaluation of several drug combinations for intraperitoneal anaesthesia in adult male rats

Hajighahramani, S. and Vesal, N.*

Department of Clinical Sciences, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran

***Correspondence:** N. Vesal, Department of Clinical Sciences, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran. E-mail: nv1340@shirazu.ac.ir

(Received 26 Aug 2006; revised version 17 Oct 2006; accepted 30 Oct 2006)

Summary

The objective of this study was to evaluate several drug combinations for intraperitoneal anaesthesia in laboratory rats. Following dose determination of anaesthetics in a pilot study, 45 healthy adult male rats were randomly assigned in 9 treatment groups and received propofol (alone or in combination with xylazine, midazolam, or ketamine) or ketamine (in combination with xylazine, midazolam, acepromazine, acepromazine-xylazine, or midazolam-xylazine). Heart and respiratory rate, induction and total sleep times, duration of surgical anaesthesia and walking time were measured. Complete immobility and loss of righting reflex were observed within 12 min in all groups. Induction of anaesthesia was significantly longer following XP compared to other groups. Surgical anaesthesia was induced in all rats receiving XK, AXK and MXK, while propofol alone, MK and KP were associated with surgical anaesthesia in 2, 3 and 4 rats, respectively. Other combinations did not produce surgical anaesthesia. Duration of surgical anaesthesia was longest with MXK and shortest with MK. In conclusion, the most effective drug combinations, which result in longer duration of surgical anaesthesia, were AXK and MXK. Although the degree of analgesia produced by IP propofol is sufficient for restraint and non-painful procedures, the combination of ketamine-propofol can produce surgical anaesthesia in the rat.

Key words: Anesthesia, Intraperitoneal, Propofol, Ketamine, Rat

Introduction

Appropriate anaesthesia is necessary for surgical procedures in rats. Although inhalation anaesthetics are generally safer than injectable anaesthetics, their use may be limited by lack of equipment, facilities, or expertise of the anaesthetists. The small diameter of the airways and the anatomy of the oropharynx prevent routine endotracheal intubation in rats (Flecknell, 1996; Thurmon *et al*., 1996). Therefore, injectable anaesthetic agents tend to be more commonly used in the laboratory setting (Flecknell, 1996; Hedenqvist and Hellebrekers, 2003).

Intraperitoneal (IP) injection has been commonly used in laboratory rats because it requires minimal skill and does not result lesions or sign of pain when irritating drugs are administered. Intramuscular (IM) injection of irritant drugs may cause swelling and lameness in the injected limb, which may eventually result in self-mutilation (Smiler *et al*., 1990). For this reason, the intramuscular route is avoided in small rodents. Intravenous (IV) injection into the tail veins is possible if rat-restraining cage is used. This method of drug administration is difficult for inexperienced persons. In very small species, such as mice and rats, the intravenous route for routine drug administration is rarely practical and drugs are generally administered subcutaneously or intraperitoneally. IP injection has the advantage of being both simple and also causes a minimum of distress to the rat (Flecknell, 1996).

A wide variety of different injectable anaesthetic techniques have been used for induction of anaesthesia in the rat, including pentobarbital, thiopental, ketamine and tiletamine (Flecknell, 1996; Thurmon *et al*., 1996). Ketamine alone does not provide sufficient analgesia and muscle relaxation but its combination with sedative agents (α_2-agonists, phenothizines and benzo-diazepines) is widely used in rodents (Flecknell, 1996; Hedenqvist and

Hellebrekers, 2003). Ketamine is well absorbed following IP and IM injection in rats, but IM injection is painful and irritating; thus IP injection is preferred. Propofol is a sedative-hypnotic, nonbarbiturate agent characterized by a rapid onset and short duration of action with minimal analgesic action at a subanaesthetic dose. Because of its rapid redistribution and metabolism, propofol has been used as an intravenous anaesthetic agent for induction and maintenance of anaesthesia in various species (Thurmon *et al*., 1996). Intravenous administration of propofol (10 mg/kg) produces surgical anaesthesia in fentanyl-fluanisone premedicated rats (Brammer *et al*., 1993). Clearance of propofol is rapid in rat, ranging from 30 to 80 ml/kg/min (Cockshott *et al*., 1992). In the present study, the lipid emulsion formulation of propofol, alone or in combination with other drugs, was evaluated as an intraperitoneal induction agent. The objective of this study was to determine the induction dose of several anaesthetic regimens and compare the suitability and clinical effects of drug combinations for induction of anaesthesia following intraperitoneal administration in unpremedicated rats.

Materials and Methods

Forty-five healthy adult male Sprague-Dawley rats weighing 237 ± 27 g (mean ± SD) were used in this study. They were group-housed in solid floored caging in a temperature regulated room (22 ± 2°C) with a 12/12 hr light/dark cycle and received a commercial pellet diet and water ad libitum. Sawdust was used as bedding. The animal's weight in gram was determined individually before drug administration. Rats were allowed an acclimation period of at least 4 weeks prior to the beginning of the study. Regular handling and restraint was performed in order to habituate the animal to the procedure. They were not fasted before the administration of medication in order to reduce the risk of hypoglycaemia and dehydration (Flecknell, 1996; Wixson and Smiler, 1997).

Pilot study

The objective of pilot study was to establish appropriate dose rates of anaesthetic agents. Different doses of each drug or drug combinations were studied using an up-and-down procedure (data not shown) (Aeschbacher and Webb, 1993). At least four rats were used for each drug dose. All anaesthetics were administered by the IP route.

Main study

The quality of anaesthesia induced by each drug/drug combinations with the dose that was effective during pilot study was evaluated. Two weeks following the completion of the pilot study, rats were randomly assigned to one of nine treatment groups and received propofol (Lipuro 1%-Propofol 10 mg/ml, B. Braun Melsungen, Germany) (alone or in combination with xylazine (20 mg/ml, Alfasan, Woerden, Holland), midazolam (ormicum, 5 mg/ml, Hoffman-La Roche Ltd, Basel, Switzerland) or ketamine (00 mg/ml, Aesculaap, Boxtel, Holland)) or ketamine (in combination with xylazine, midazolam, acepromazine (Castran, 15 mg/ml, interchemie, Holland), acepromazine-xylazine, or midazolam-xylazine) intraperitoneally. Details of the anaesthetic protocols are given in Table 1. There were 5 rats in each group. The rat was

Table 1: Treatment groups

Groups	Drug/Drug combinations
1- Group-P	Propofol (100 mg/kg)
2- Group-XP	Xylazine (1 mg/kg)-Propofol (90 mg/kg)
3- Group-MP	Midazolam (2 mg/kg)-Propofol (50 mg/kg)
4- Group-KP	Ketamine (40 mg/kg)-Propofol (60 mg/kg)
5- Group-XK	Xylazine (3 mg/kg)-Ketamine (75 mg/kg)
6- Group-MK	Midazolam (5 mg/kg)-Ketamine (75 mg/kg)
7- Group-AK	Acepromazine (2.5 mg/kg)-Ketamine (75 mg/kg)
8- Group-AXK	Acepromazine (2.5 mg/kg)-Xylazine (3 mg/kg)-Ketamine (75 mg/kg)
9- Group-MXK	Midazolam (3 mg/kg)-Xylazine (3 mg/kg)-Ketamine (75 mg/kg)

held in head down position in an attempt to avoid penetration of viscera and injection was made into the lower left abdominal quadrant, using a 25 gauge needle. Because the volume of drug to be injected is often quite small in rats, ketamine, xylazine, midazolam and acepromazine were diluted with normal saline (0.9%), to provide a suitable volume for dosing (0.2-0.3 ml/rat). Drug combinations were mixed in a syringe immediately prior to administration and given in one injection to minimize handling stress.

Following loss of righting reflex, rats were laid in dorsal recumbency. Respiratory rate (counted by observing thoracic movement), ECG (Burdick Electro-cardiograph, Sylmar, CA, USA) (lead I, using hypodermic needle electrode, paper speed 50 mm/sec) and heart rate (counted from ECG recording) recorded at the 5, 10, 15, 20, 25, 30, 40, 50 and 60-min post-injection intervals until the righting reflex returned. Rats breathed room air throughout the anaesthesia. Since rats show a marked tachypnea when handled or disturbed, an estimate of normal respiratory rate was made by observing 16 of the animals used in the study, while the animals remained undisturbed in their home cages.

Rectal temperature was monitored using digital thermometer inserted at least 3 cm into the rectum at the 5-min post-injection intervals. Body temperature was maintained at 37-38°C during anaesthesia using a heat lamp. A thermometer was placed next to the animal to avoid excessive heat application. When the eyes remained open or partially open during anaesthesia (especially with ketamine combinations), sterile saline was applied to the eyes to prevent corneal drying.

The depth of anaesthesia was monitored by use of the withdrawal reflexes (pedal withdrawal, lip and tail pinch reflexes), and righting reflex. The withdrawal reflexes were assessed by pinching the interdigital of the hind-foot, the lower lip (with an atraumatic plastic forceps), or distal part of the tail (with the thumb and index finger) every 5 min after loss of righting reflex until righting reflex was regained. All reflex tests were carried out and assessed by the same operator. Responses were scored on a scale of 0-4, with complete reflex absent scoring 0 and a strong withdrawal response scoring 4 (Hedenqvist *et al*., 2000). Induction time (time to loss of righting reflex), total sleep time (duration of loss of righting reflex), duration of surgical anaesthesia (the period of loss of pedal withdrawal reflex) and walking time (duration from loss of righting reflex until ability to walk) were recorded. Muscle relaxation was assessed by recording the presence or absence of skeletal muscle tone.

Following drug trials, eight rats undergoing propofol anaesthesia were used for gross and histologic evaluation of acute and chronic inflammation 3, 12 and 15 days after drug administration. Rats were humanely euthanized with ether overdose and a necropsy was performed. All abdominal viscera were examined specifically for evidence of adhesion formation as well as any other gross indication of inflammation. Peritoneum, abdominal muscle, liver, intestine, and spleen were prepared for histologic evaluation.

Statistical analysis

Statistical analysis of parametric data (induction and recovery times) was performed using one-way analysis of variance (ANOVA) followed by Duncan's test when appropriate. A repeated measure analysis of variance with time and treatment as factor was used to compare physiologic values (heart and respiratory rates). Pain scores were analysed using Kruskal-Wallis test. All results are expressed as mean ± SEM and differences were considered significant at $p<0.05$. Statistical analyses were performed using SPSS Version 11.5 for Windows (MicroMaster Inc., Richboro, PA, USA).

Results

All drugs or drug combinations produced complete immobility and loss of righting reflex within 12 min after IP injection. Induction of anaesthesia was very fast with midazolam-ketamine and very slow with xylazine-propofol (Table 2). There was no significant difference between midazolam-ketamine (MK), midazolam-

Table 2: Induction time, duration of surgical anaesthesia, total sleep time and walking time (min) after IP injection of anaesthetic drug(s) in rats (Mean ± SEM (n))

Drug (s)	Induction time	Duration of SA	Sleep time	Walking time
Pro	6.6±0.4^{b}	27.5±2.5 (2)	48.2±9.3^{bc}	51.6±9.9^{abc}
Xyl-Pro	11.6±0.9^{a}	-	24.8±6.7^{a}	26.2±6.6^{a}
Mid-Pro	4.8±0.8^{bc}	-	34.8±8.0^{ab}	38.4±9.2^{ab}
Ket-Pro	3.4±0.5^{cd}	32.5±7.5^{ab} (4)	44.0±5.7^{ab}	49.6±5.8^{abc}
Xyl-Ket	2.6±0.2^{d}	17.0±3.4^{a} (5)	41.0±4.4^{ab}	56.0±4.9^{bc}
Mid-Ket	1.8±0.2^{d}	16.7±7.6^{a} (3)	47.2±2.5^{bc}	60.4±5.6^{bc}
Acp-Ket	3.4±0.5^{cd}	-	25.6±4.3^{a}	41.2±8.1^{ab}
Acp-Xyl-Ket	3.0±0.8^{cd}	25.0±6.5^{ab} (5)	51.2±7.4^{bc}	67.6±10.5^{c}
Mid-Xyl-Ket	2.0±0.3^{d}	37.0±6.4^{b} (5)	65.8±8.0^{c}	73.2±10.0^{c}

Different letters in the same columns indicates significant different (P≤0.05) between the groups

xylazine-ketamine (MXK), xylazine-ketamine (XK), acepromazine-xylazine-ketamine (AXK) and acepromazine-ketamine (P≥0.05). The time of loss of the righting reflex was significantly longer following xylazine-propofol (XP) compared to other groups (P≤0.05). All drug combinations provided adequate muscle relaxation as judged by complete loss of skeletal muscle tone.

Withdrawal reflexes (pedal withdrawal, lip and tail pinch reflexes) were used to assess depth of anaesthesia (Table 3). The lip pinch reflex was lost first followed by the tail reflex. The response to pinching the toe disappeared at deeper levels of anaesthesia than the tail pinch reflex. Recovery of reflexes was in the reverse order. Absence of pedal withdrawal reflex was considered as the onset of surgical anaesthesia (Flecknell, 1996). The pedal withdrawal score was significantly lower in MXK and AXK groups (P≤0.05).

Surgical anaesthesia was induced in all rats receiving XK, AXK and MXK (Table 2). Administration of propofol alone, MK and KP was associated with surgical anaesthesia in 2, 3 and 4 rats, respectively. Xylazine-propofol (XP), midazolam-propofol (MP) and acepromazine-ketamine (AK) were not able to produce surgical anaesthesia and none of the rats in these groups lost the pedal withdrawal reflex. Duration of surgical anaesthesia was longest with MXK and shortest with MK. The addition of midazolam to xylazine-ketamine combination significantly (P≤0.05) increased the duration of surgical anaesthesia (37.0 ± 6.4 vs 17.0 ± 3.4 min) and sleep time (65.8 ± 8.0 vs 41.0 ± 4.4 min). Sleep time was significantly longer for MXK compared to AK, XK, KP, MP and XP (P≤0.05). Walking time was the shortest with XP and the longest with MXK (Table 2). AXK and MXK groups had significantly longer walking times compared to XP, MP and AK groups (P≤ 0.05).

Table 3: Lip, tail and pedal withdrawal scores after IP injection of anaesthetic drug(s) in rats (Mean ± SEM)

Drug(s)	Lip pinch	Tail pinch	Pedal pinch
Pro	2.0±0.3^{d}	2.6±0.2^{abc}	2.6±0.3^{a}
Xyl-Pro	1.9±0.3^{d}	2.1±0.3^{ab}	3.4±0.1^{ab}
Mid-Pro	2.4±0.3^{de}	3.1±0.2^{ac}	2.8±0.3^{ab}
Ket-Pro	0.5±0.2^{abc}	2.2±0.2^{abc}	1.2±0.1^{d}
Xyl-Ket	0.3±0.1^{abc}	1.4±0.2^{e}	1.9±0.3^{c}
Mid-Ket	0.8±0.3^{bc}	2.7±0.2^{abc}	1.9±0.2^{c}
Acp-Ket	2.9±0.3^{de}	3.8±0.1^{f}	3.5±0.2^{b}
Acp-Xyl-Ket	0.2±0.2^{abc}	0.6±0.2^{d}	1.2±0.2^{de}
Mid-Xyl-Ket	0.0±0.0^{ab}	0.5±0.2^{d}	0.6±0.2^{de}

Different letters in the same columns indicates significant different (P≤0.05) between the groups

Full recovery in animals given P, XP and MP was prolonged. Although the rats regained the righting reflex within a relatively short period, they usually appeared sedated and unresponsive to sound for an extended time. Duration of sedation was 59.8 ± 5.5, 38.6 ± 7.3 and 19.4 ± 4.3 min in propofol, MP and XP groups, respectively. The mean sedation time was significantly longer in the propofol group compared to MP and XP groups (P≤0.05). In contrast, rats in other treatment groups were relatively alert and seemed to arouse easily. Animals received propofol may be aroused from apparently profound sedation by direct stimulation. It is interesting to note that sedation was not observed during recovery

in rats receiving ketamine-propofol.

The resting respiratory rate of rats, observed undisturbed, was recorded as 122 ± 3 breaths per minute. Respiratory rate reduced markedly following induction of anaesthesia in all groups except MK and AK (Table 4). Lowest value for respiratory rate occurred at 20 min after XP injection (59 ± 3 breaths/min). Overall, groups received propofol alone or in combination had lower respiratory rate compared to ketamine combination groups. Respiratory rate was significantly lower in PX, P and PM groups compared to MK and AK groups ($P \leq 0.05$). At 10 min post-injection, rats in MK and AK groups had significantly higher respiratory rate compared to other groups. In xylazine-ketamine group, there was a significant reduction in the respiratory rate at 10, 15 and 20 min post-injection, compared to MK and AK groups ($P \leq 0.05$). No periods of apnea or cyanosis occurred in any groups.

Heart rate was lower in animals receiving xylazine (i. e., AXK, XK, MXK and XP groups) (Table 5). Lowest value for heart rate observed at 10 min after XK injection (250 ± 10 beats/min). Heart rate was significantly higher in MP and MK groups compared to AXK, XK and MXK groups ($P \leq 0.05$). Heart rate was regular and ECG revealed no arrhythmias in any groups.

Increased urine production was noted in rats given xylazine. Frequent urination was observed during anaesthesia in 4, 3 and 2 rats in XK, MXK and AXK groups, respectively. There was no excitement during induction and no adverse side effects were observed. None of the rats died during or after anaesthesia. Rats receiving propofol had no visible lesion on gross examination. There was no histologic evidence of inflammation in the peritoneum or in the serosa of abdominal organs.

Discussion

In order to avoid gender effects, only male rats were used in this study. It has been reported that female rats are more sensitive to pentobarbital, ketamine and

Table 4: Respiratory rate (breaths/min) after IP injection of anaesthetic drug(s) in rats (Mean ± SEM)

Drug(s)	Time (minutes)							
	5	10	15	20	25	30	40	50
Pro	82±8^{ab}	78±4^{a}	70±4^{a}	71±5^{a}	71±4^{ab}	68±4	71±10	-
Xyl-Pro	77±11^{ab}	72±7^{a}	67±5^{a}	59±3^{a}	-	-	-	-
Mid-Pro	73±2^{a}	70±6^{a}	68±5^{a}	69.6±3^{a}	73±2^{ab}	-	-	-
Ket-Pro	97±8^{abc}	74±67^{a}	78±9.5^{ab}	82±12^{ab}	109±22^{bc}	131±30	95±32	-
Xyl-Ket	92±10^{abc}	66±3^{a}	67±3^{a}	68±3^{a}	88±11^{ab}	93±12	121±16	-
Mid-Ket	116±18^{cd}	101±8^{b}	100±6^{b}	101±9^{b}	112±13^{bc}	116±14	128±15	150±5
Acp-Ket	129±30^{d}	118±38^{b}	128±36^{c}	148±10^{c}	134±7^{c}	163±12	-	-
Acp-Xyl-Ket	103±9^{bcd}	73±7^{a}	66±7^{a}	69±10^{a}	88±16^{ab}	103±25	130±39	130±39
Mid-Xyl-Ket	85±5^{ab}	62±10^{a}	64±10^{a}	66±10^{a}	72±14^{ab}	74±13	81±11	96±22

Different letters in the same columns indicates significant different ($P \leq 0.05$) between the groups. (-) not determined due to animal's recovery

Table 5: Heart rate (beats/min) after IP injection of anaesthetic drug(s) in rats (Mean ± SEM)

Drug(s)	Time (minutes)							
	5	10	15	20	25	30	40	50
Pro	366±19^{b}	326±13^{ab}	316±12^{abc}	304±13^{ab}	312±15^{abcd}	312±22	293±29	275±5
Xyl-Pro	308±12^{c}	310±8^{ad}	298±9^{abcf}	290±15^{a}	-	-	-	-
Mid-Pro	422±22^{ab}	404±27^{f}	398±24^{e}	416±25^{c}	385±17^{e}	-	-	-
Ket-Pro	396±18^{ab}	342±18^{abe}	324±16^{bcd}	316±17^{ab}	360±37^{cde}	368±37	333±38	-
Xyl-Ket	258±6^{c}	250±10^{c}	264±14^{af}	272±10^{a}	284±8^{ab}	280±11	277±11	-
Mid-Ket	412±16^{ab}	374±14^{bef}	350±24^{cde}	354±24^{b}	370±30de	360±28	392±26	-
Acp-Ket	388±23^{ab}	380±27^{ef}	372±29de	345±3^{b}	343±8^{bcde}	357±12	-	-
Acp-Xyl-Ket	290±15^{c}	258±7^{c}	260±8^{f}	266±10^{a}	262±11^{a}	258±11	260±11	247±9
Mid-Xyl-Ket	288±6^{c}	274±7^{cd}	274±7^{abf}	276±14^{a}	272±17^{ab}	274±15	266±16	270±18

Different letters in the same columns indicates significant different ($P \leq 0.05$) between the groups. (-) not determined due to animal's recovery

medetomidine-ketamine combination than male rats (Green, 1979; Nevalainen *et al*., 1989; Hall *et al*., 2001). Basal metabolic rate is about 7% higher in the males compared to the non-pregnant female rats (Green, 1979).

Due to poorly accessible peripheral vessels for IV injection, the IP route of drug administration is probably the most popular parenteral method of drug delivery in rodents (Flecknell, 1996; Wixson and Smiler, 1997). In this method drugs are administered into a well-perfused space with a large peritoneal surface for absorption. When used intramuscularly, the acid pH of ketamine has been associated with muscle damage and nerve irritation and should be given preferentially through the IP route (Smiler *et al*., 1990). Ketamine, in combination with xylazine or diazepam, is shown to be non-irritating and suitable for IP injection in the laboratory rat (Wixson *et al*., 1987a). In the present study, intraperitoneal injection of propofol caused no gross or microscopic lesions in the peritoneal cavity. Unlike thiopental, propofol does not produce tissue damage following accidental perivascular or intraarterial injection (Glen, 1980; Thurmon *et al*., 1996). Pain on injection has been reported following IV propofol administration in human, but not in dog or cat (Glen, 1980). In the present study, rats receiving propofol showed no sign of pain, i. e., struggling/vocalization, following IP injection.

The peritoneum is a continuous semipermeable membrane between fluid of the peritoneal cavity and fluid of extracellular water. It covers the internal abdominal organs (visceral peritoneum) and the abdominal wall (parietal peritoneum) and forms the mesentery (Getty, 1975). The surface area of the peritoneum is very large and drug absorption occurs mainly across peritoneal capillaries. Peritoneal lymphatics are also responsible for the absorption of fluid and drugs. Compared to IV injection, absorption is about four times slower following IP injection but faster than from an IM injection (Waynforth, 1995). Slow rate of absorption may result in low plasma concentration.

Some of the active drug absorbed from the peritoneal cavity must pass through the liver by the portal system and undergo hepatic metabolism before reaching the systemic circulation (first-pass effect) (Waynforth, 1995; Hedenqvist and Hellebrekers, 2003). Therefore, the systemic bioavailability of the drug after intraperitoneal injection will be lower compared to SC or IM routes and larger doses are necessary to attain the same therapeutic blood levels. An increased dose by approximately 50-75% has been recommended for intraperitoneal injection relative to that needed with IM and SC routes (Hedenqvist and Hellebrekers, 2003).

Although all drug or drug combinations produced loss of righting reflex, propofol alone or in combination with xylazine or midazolam did not produce satisfactory surgical anaesthesia in rats. When propofol was given by IP injection, righting reflex was lost within 6-8 min and lasted 48.2 ± 9.3 min. Complete recovery required additional 59.8 ± 5.5 min.

Propofol at a dose of 10-25 mg/kg IV has been recommended for repetitive bolus administration in unpremedicated rat (Glen, 1980; Brammer *et al*., 1993). A single dose of 7.5-15 mg/kg IV propofol produces a short duration of anaesthesia and should be followed by continuous infusion of 44-45 mg/kg/hr. (Glen, 1980; Flecknell, 1996). Induction of anaesthesia is very rapid following IV administration of propofol and usually occurs in less than one minute. Intramuscular administration of propofol to rabbits, even at relatively high doses (60 mg/kg), results only in sedation after 12 min (Glen, 1980). In cat receiving 36 mg/kg propofol IM, loss of righting reflex occurred at 18 min post-injection and anaesthesia lasted for one hr. Intramuscular injection produces sedation only (Glen, 1980). In the present study, mean induction time was 6.6 ± 0.4 min following 100 mg/kg propofol IP; light and surgical anaesthesia were observed in 3 and 2 rats, respectively. These results indicate faster propofol absorption after IP injection compared to IM route. Hasan and Woolley (1994) demonstrated that intraperitoneal subanaesthetic dose of propofol (25-50 mg/kg) has a marked anticonvulsant effect in the rat. The effect of tumour necrosis factor alpha on anaesthesia time has been studied in rats given

intraperitoneal propofol (80 mg/kg) (Yasuda *et al*., 2002). The systemic availability of propofol following IP administration has not been determined. At present, propofol is available at a concentration of 10 mg/ml, therefore, relatively large volume of IP propofol (2-3 ml per rat) is required to produce anaesthesia, which can cause discomfort to the animal.

Advantages of propofol are its rapid onset of action after IV administration, short duration of action, lack of accumulation after repetitive administration and rapid recovery. Propofol may be used as a continuous infusion agent due to minimal cumulative effect (Hedenqvist and Hellebrekers, 2003). Its disadvantages include poor analgesia, respiratory depression and the requirement for IV access. Propofol is non-irritant when injected perivascularly (Thurmon *et al*., 1996). Iwasaki *et al*. (1991) demonstrated that intraperitoneal administration of propofol (50-100 mg/kg) has no effect on tail-flick test in rats. The addition of xylazine or midazolam to propofol did not improve analgesia in the present study.

Induction time with xylazine-propofol (11.6 ± 0.9 min) was significantly longer when compared to propofol alone (5.6 ± 0.7 min). The reason for this difference is not clear and probably the concurrent administration of xylazine could have interfered with peritoneal absorption of propofol that is available as an aqueous emulsion. Alpha$_2$-agonists have a direct local vasoconstriction effects that may reduce drugs absorption. Spinal cord blood flow and metabolic rate reduces and spinal anaesthesia prolongs following subarachnoid administration of clonidine, an α_2-adrenoceptor agonist, in rats (Crosby *et al*., 1990).

Medetomidine, a more potent α_2 agonist, in combination with IV propofol has been reported for short duration anaesthesia in rabbit (Hellebrekers *et al*., 1997). The α_2-adrenoceptor agonist, clonidine, increases propofol-induced sleep time in rat (Kushikata *et al*., 2002). Propofol was administered IP 60 min after clonidine injection. In the present study, sleep time was shorter in xylazine-propofol group compared to propofol alone. The lower dose of propofol used in XP group (90 vs 100 mg/kg) may partly explain the reduction in sleep time. In the previous study, clonidine needed a 60-min lag time to reach its full effect (Kushikata *et al*., 2002), while in our study xylazine and propofol have been given as a single IP injection.

Animals given propofol alone or in combination with xylazine or midazolam remained sedated for prolonged periods. This may be attributed to the slow continued absorption of propofol from peritoneal cavity. It is interesting to note that sedation was not observed during recovery in rats receiving ketamine-propofol, probably due to CNS stimulant properties of ketamine. The mechanisms involved in dissociative anaesthesia are different from those of barbiturates and inhalation anaesthetics and ketamine-induced seizure activity has been reported in various species (Fish, 1997).

Respiratory effects of propofol include a dose-dependent decrease in minute volume and respiratory arrest may follow after high doses. Rapid IV administration of propofol in rodents causes 5-10 sec of apnea and hypotension (Flecknell, 1996). In the present study, propofol administration was not associated with apnea. However, respiratory depression was observed in rats given propofol alone or in combination with other agents. Saiki *et al*. (2003) demonstrated that decrease in ventilation during propofol anaesthesia in rats is mainly due to the reduction of metabolic rate. Propofol administration resulted in depression of respiratory function; therefore, it is strongly recommended that oxygen be always administered when this agent is used to provide anaesthesia in rats. The resting respiratory rate of rats was 122 ± 3 breaths/min, which is similar to the previously reported normal range (70-150 breaths/min) (Green, 1979). In the present study, the animals were breathing room air and despite the decrease in respiratory rate, cyanosis or severe respiratory depression was not observed. In general, respiratory rate in rat should not decrease below 60 breaths/min, which indicates serious respiratory depression.

Ketamine has been widely used in combination with sedative drugs in rodents. It is well absorbed following IP and IM

injection in rats, but IM injection is painful and may result in tissue necrosis (Smiler *et al*., 1990). During ketamine anaesthesia, the eyes may remain open, palpebral reflex is present and muscle relaxation is poor. Benzodiazepines, α_2-agonists and phenothiazine tranquillizers have been used in conjunction with ketamine to provide a more balanced state of general anaesthesia.

Ketamine/xylazine combination has been widely used to induce anaesthesia in rats (Hsu *et al*., 1986; Wixson *et al*., 1987a; Flecknell, 1996). This mixture produces a rapid onset of anaesthesia with generally good analgesia and muscle relaxation. Doses of ketamine in the range of 50-100 mg/kg and 3-10 mg/kg of xylazine provide surgical anaesthesia of short to moderate duration (Fish, 1997). Dose ratios should be adjusted based on the surgical procedure and the duration of anaesthesia required. In our study, xylazine-ketamine produced greater respiratory depression than did midazolam-ketamine or acepromazine-ketamine. This is in agreement with previous study in which diazepam-ketamine was used in rat (Wixson *et al*., 1987b).

Midazolam (a benzodiazepine) and acepromazine (a phenothiazine derivative) both produce minimal respiratory depression at clinical doses. Midazolam provides reliable sedation and muscle relaxation with minimal cardiovascular and respiratory depression in rodents (Fish, 1997). Rats received MK or AK combinations showed minimal changes in heart and respiratory rates (Tables 4 and 5). Unlike diazepam, it is water-soluble and can be mixed with other water-soluble drugs in a single syringe. Midazolam and acepromazine have no analgesic properties, but potentiates the effect of most anaesthetic agents by influencing gamma-aminobutyric acid receptors and central dopamine blockade, respectively (Fish, 1997). Midazolam and acepromazine have been used in combination with ketamine in rodents (Flecknell, 1996; Hedenqvist and Hellebrekers, 2003). In our study, 3 rats reached surgical anaesthesia in MK group, while none of the rats in AK group lost the pedal withdrawal reflex. It has been reported that MK and AK combinations produce light anaesthesia in rats (Flecknell, 1996). Gardner *et al*. (1995) documented similar results with acepromazine-ketamine combination in mice.

Combination of ketamine-propofol has been described in sheep (Correia *et al*., 1996), ponies (Nolan *et al*., 1996), horses (Edner *et al*., 2002) and cats (Ilkiw *et al*., 2003). Ketamine has been found to be additive when combined with propofol for induction of anaesthesia in human (Hui *et al*., 1995). Addition of ketamine to propofol provides hemodynamic stability and analgesia. In the present study, ketamine was added to propofol in order to improve analgesic effects. Surgery was not performed in this study and the depth of anaesthesia was evaluated by assessment of withdrawal reflexes. Pedal withdrawal reflex was judged to determine the onset and duration of surgical anaesthesia. Pedal withdrawal score in KP group was similar to AXK and MXK groups, although one rat in KP group did not reach adequate depth of anaesthesia.

Combination of ketamine, medetomidine and diazepam has been used subcutaneously in 340 rabbits undergoing orthopaedic surgery with no mortality (Mero *et al*., 1989). This combination produced complete analgesia and muscle relaxation and surgical anaesthesia of 30-60 min duration. Intraperitoneal administration of ketamine-xylazine-acepromazine combination in mice has been associated with longer duration of surgical anaesthesia and high safety margin (Arras *et al*., 2001). In the present study, acepromazine or midazolam were added to xylazine-ketamine mixture in order to prolong the duration of surgical anaesthesia and improve analgesia. MXK mixture was associated with significantly longer duration of surgical anaesthesia compared to XK combination. Pedal withdrawal score was significantly lower in AXK and MXK groups compared to XK group (Table 3).

Blood pressure was not measured in the present study. However, there were no signs of serious cardiovascular depression. Heart and respiratory rates were regular and cyanosis was not observed in any treatment groups. Heart rate is usually elevated following propofol administration in rats (Fish, 1997). However, Akine *et al*. (2001) reported a 5.5% and 18.9% reduction in

heart rate and blood pressure, respectively, following intravenous propofol (10 mg/kg) injection in chronically instrumented rat. In contrast, ketamine (10 mg/kg) increased heart rate and blood pressure by 17.7% and 30%, respectively. Ketamine usually stimulates cardiovascular function, causing an increase in heart rate and blood pressure (Thurmon *et al*., 1996). Bradycardia, sinus arrhythmias and atrioventricular block are common side effect of α_2-agonists as a result of reflex vagal activity and α_2-mediated decrease in norepinephrine release in the sympathetic nervous system (Thurmon *et al*., 1996). Although bradycardia was observed in rats receiving xylazine, it did not produce any changes in myocardial conductivity.

Polyuria observed in rats receiving xylazine is due to inhibition of antidiuretic hormone release and osmotic diuretic effect of hyperglycaemia induced by α_2-agonist agents (Hsu *et al*., 1986; Wixson *et al*., 1987a; Thurmon *et al*., 1996). Polyuria has been reported in rats given medetomidine (a more potent α_2-agonist) in combination with ketamine (Nevalainen *et al*., 1989). Alpha$_2$-agonist agents are not recommended in dehydrated or hypovolaemic animals.

In Conclusion, Ketamine-propofol, xylazine-ketamine, acepromazine-xylazine-ketamine and midazolam-xylazine-ketamine appear to be acceptable drug combinations for IP anaesthesia in the rat. Based on our results, the most effective drug combination, which resulted in adequate anaesthesia with longer duration in all rats, was midazolam-xylazine-ketamine. Acepromazine-xylazine-ketamine is another safe and effective anaesthetic combination for rat. The degree of analgesia produced by IP propofol is insufficient for surgical procedures but a combination of ketamine-propofol can produce surgical anaesthesia in the rat. Further investigation is required to evaluate the systemic bioavailability and cardiopulmonary effects of propofol when administered IP in rats.

Acknowledgement

This research was financially supported by grant No 83-VE-1755-C296 from Research Council of Shiraz University.

References

Aeschbacher, G and Webb, AI (1993). Propofol in rabbits, 1. Determination of an induction dose. Lab. Anim. Sci., 43: 324-327.

Akine, A; Suzuka, H; Hayashida, Y and Kato, Y (2001). Effects of ketamine and propofol on autonomic cardiovascular function in chronically instrumented rat. Auton. Neurosci-Basic. 87: 201-208.

Arras, M; Autenried, P; Rettich, A; Spaeni, D and Rulicke, T (2001). Optimization of intraperitoneal injection anesthesia in mice: drugs, dosages, adverse effects and anesthetic depth. Comparative Med., 51: 443-456.

Brammer, A; West, CD and Allen, SL (1993). A comparison of propofol with other injectable anaesthesia in a rat model for measuring cardiovascular parameters. Lab. Anim., 27: 250-257.

Cockshott, ID; Douglas, EJ; Plummer, GF and Simons, PJ (1992). The pharmacokinetics of propofol in laboratory animals. Xenobiotica. 22: 369-375.

Correia, D; Nolan, AM and Reid, J (1996). Pharmacokinetics of propofol infusion, either alone or with ketamine in sheep premedicated with acepromazine and papaveretum. Res. Vet. Sci., 60: 213-217.

Crosby, G; Russo, MA; Szabo, MD and Davies, KR (1990). Subarachnoid clonidine reduces spinal cord blood flow and glucose utilization in conscious rats. Anesthesiology. 73: 1179-1185.

Edner, A; Nyman, G and Essen-Gustavsson, B (2002). The relationship of muscle perfusion and metabolism with cardiovascular variables before and after detomidine injection during propofol-ketamine anesthesia in horses. Vet. Anaesth. Analg., 29: 182.

Fish, RE (1997). Pharmacology of injectable anesthetics. In: Kohn, DF; Wixon, SK; White, WJ and Benson, GJ (Eds.), *Anesthesia and analgesia in laboratory animals*. (1st. Edn.), San Diego, Academic Press. PP: 1-28.

Flecknell, P (1996). *Laboratory animal anaesthesia*. 2nd. Edn., London, UK, Elsevier Academic Press. PP: 161-168.

Gardner, DJ; Davis, JA; Weina, PJ and Theune, B (1995). Comparison of tribromoethanol, ketamine/acetylpromazine, telazol/xylazine, pentobarbital and methoxyflurane anesthesia in HSD:ICR mice. Lab. Anim. Sci., 45: 199-224.

Getty, R (1975). *Sisson and Grossman's anatomy of domestic animals*. 5th. Edn., Vol.

1. Philadelphia, W. B. Saunders Co., PP: 84-103.

Glen, JB (1980). Animal studies of the anesthetic activity of ICI 35 868. Br. J. Anaesth., 52: 731-742.

Green, CJ (1979). *Animal anaesthesia*. 1st. Edn., London, Laboratory Animal LTD. PP: 237-241.

Hall, LW; Clarke, KW and Trim, CM (2001). Anaesthesia of birds, laboratory animals and wild animals. *Veterinary anaesthesia*. (10th. Edn.), London, W. B. Saunders Co., PP: 113-131, 463-479.

Hasan, ZA and Woolley, DE (1994). Comparison of the effects of propofol and thiopental on the pattern of maximal electroshock seizures in the rat. Pharmacol. Toxicol., 74: 50-53.

Hedenqvist, P and Hellebrekers, LJ (2003). Laboratory animal analgesia, anesthesia, and euthanasia. In: Hau, J and Van Hoosier, GL (Eds.), *Handbook of laboratory animal science*. (2nd. Edn.), CRC Press, Boca Raton. PP: 413-455.

Hedenqvist, P; Roughan, JV and Fleknell, PA (2000). Effects of repeated anesthesia with ketamine/medetomidine and preanesthetic administration of buprenorphine in rats. Lab. Anim., 34: 207-211.

Hellebrekers, LJ; de Boer, EJ; van Zuylen, MA and Vosmeer, H (1997). A comparison between medetomidine-ketamine and medetomidine-propofol anaesthesia in rabbits. Lab. Anim., 31: 58-69.

Hsu, WH; Bellin, SI; Dellmann, HD and Hanson, CE (1986). Xylazine-ketamine induced anesthesia in rats and its antagonism by yohimbine. J. Am. Vet. Med. Assoc., 189: 1040-1043.

Hui, TW; Short, TG; Hong, W; Suen, T; Gin, T and Plummer, J (1995). Additive interaction between propofol and ketamine when used for anesthesia induction in female patients. Anesthesiology. 82: 641-648.

Ilkiw, JE; Pascoe, PJ and Tripp, LD (2003). Effect of variable-dose propofol alone and in combination for total intravenous anesthesia in cats. Am. J. Vet. Res., 64: 907-912.

Iwasaki, H; Collins, JG; Namiki, A; Yamasawa, Y; Omote, K; Omote, T and Matsumoto, M (1991). Comparison of the effect of propofol and that of pentobarbital on behavioral responses to somatic and visceral stimuli in rats. Masui. 40: 1308-1313.

Kushikata, T; Hirota, K; Yoshida, H; Kubota, T; Ishihara, H and Matsuki, A (2002). Alpha-2 adrenoceptor activity affects propofol-induced sleep time. Anesth. Analg., 94: 1201-1206.

Mero, M; Vainionpaa, S; Vasenius, J; Vihtonen, K and Rokkanen, P (1989). Medetomidin-Ketamine-Diazepam anesthesia in the rabbit. Acta Vet. Scand., 85: 135-137.

Nevalainen, T; Pyhala, L; Voipio, H-M and Virtanen, R (1989). Evaluation of anaesthetic potency of medetomidine-ketamine combination in rats, guinea pigs and rabbits. Acta Vet. Scand., 85: 139-143.

Nolan, A; Reid, J; Welsh, E; Flaherty, D; McCormack, R and Monteiro, AM (1996). Simultaneous infusion of propofol and ketamine in ponies premedicated with detomidine: a pharmacokinetic study. Res. Vet. Sci., 60: 262-266.

Saiki, C; Kamio, T; Furuya, H and Matsumoto, S (2003). Ventilation and metabolism during anesthesia in rats. Can. J. Physiol. Pharmacol., 81: 9-13.

Smiler, KL; Stein, S; Hrapkiewicz, KL and Hiben, JR (1990). Tissue response to intramuscular and intraperitoneal injection of ketamine and xylazine in rats. Lab. Anim. Sci., 40: 60-64.

Thurmon, JC; Tranquilli, WJ and Benson, GJ (1996). *Lumb and Jones' veterinary anesthesia*. 3rd. Edn., Williams and Wilkins, Baltimore. PP: 210-240, 686-735.

Waynforth, HB (1995). General aspects of the administration of drugs and other substances. In: Tuffery, AA (Ed.), *Laboratory animals: an introduction for experimenters*. (2nd. Edn.), John Wiley and Sons, Chichester. PP: 295-319.

Wixson, SK and Smiler, KL (1997). Anesthesia and analgesia in rodents. In: Kohn, DF; Wixson, SK; White, WJ and Benson, GJ (Eds.), *Anesthesia and analgesia in laboratory animals*. San Diego, Academic Press, Chapter 9: 165-203.

Wixson, SK; White, WJ; Hughes, HC; Lang, CM and Marshall, WK (1987a). A comparison of pentobarbital, fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam anesthesia in adult male rats. Lab. Anim. Sci., 37: 726-730.

Wixson, SK; White, WJ; Hughes, HC; Lang, CM and Marshall, WK (1987b). The effects of pentobarbital, fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam on arterial blood pH, blood gases, mean arterial blood pressure and heart rate in adult male rats. Lab. Anim. Sci., 37: 736-742.

Yasuda, T; Takahashi, S and Matsuki, A (2002). Tumor necrosis factor-alpha reduces ketamine and propofol induced anesthesia time in rats. Anesth. Analg., 95: 952-955.