A New Protocol of Anesthesia Using Thiopental, Diazepam and Xylazine in White New Zealand Rabbits

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Abstract: The sedative and anesthetic effects of diazepam (D), xylazine (X), thiopental (T) individually and their combinations (DX, DT, XT & DXT) were evaluated in White New Zealand rabbits. The quality of surgical anesthesia obtained from experiment 1 was tested by performing two different surgeries (Vasectomy and embryo transfer; experiment 2). Thirty two growing females and eight adult rabbits (experiment 2) were used in this study. Rabbits were injected with physiological saline, D (2.5 mg/kg), X (10 mg/kg), T (20 mg/kg) or one of the combinations (DX, XT or DXT). Rectal temperature, respiration and heart rates were recorded before and 10 min after injection and the degree and duration of sedation and anesthesia were monitored. Physiological saline had no effect on rectal temperature, respiration and heart rates. Rectal temperatures were increased following thiopental administration (P<0.05). With all treatments, rabbits exhibited depressed respiration and decreased heart rates after injection, with the exception of T which initiated an increased heart rate (P<0.01). Rabbits injected with X, T, D, DT or DX exhibited different degrees of sedation. Surgical anesthesia was obtained only after injection of XT and DXT combinations. D injection significantly prolonged the duration of anesthesia when given with XT combination (P<0.01). The anesthetic selected as superior from this comparison, DXT was used to anesthetise eight rabbits to perform either vasectomy or embryo transfer. The duration and depth of anesthesia was sufficient for performing the surgeries with minimal complications and safe recovery.

Key words: Diazepam, Xylazine, Thiopental, Rabbits, Anesthesia

INTRODUCTION

Anesthesia is the provision of relief from pain during surgery or other procedures likely to cause pain. The proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative. Although the use of anesthetics and analgesics for sample collection and surgical interventions is frequent in research and veterinary practice, professional judgement is required to select appropriate agents to avoid cardiovascular and pulmonary damage (Flecknell et al., 1983; Peeters et al., 1988; Borkowski et al., 1990) Safe, effective and reversible anesthesia requires the selection of a suitable agent(s), use of an appropriate administrative technique and accurate dosing. Both inhalant and injectable anesthetics are used in mammals and birds (Conny and Maud, 2005 and Udegbunam and Adetunji, 2007) Inhalant anesthetics are easier to administer and allow more control of the depth of anesthesia, but require expensive equipment for application. Injectable anesthetics are often preferred because they can be used without special equipment.

In veterinary medicine, thiopental is an ultra short acting barbiturate used for the induction of general anesthesia. Thiopental possesses poor analgesic and muscle relaxant properties and is usually used in combination with drugs such as xylazine and/or diazepam to improve analgesia and muscle relaxation.

In 1962, xylazine (α2 agonist) was first developed as an antihypertensive agent in humans (Greene and Thurmon, 1988). Subsequent studies revealed that xylazine induced sedation and analgesia in animals and could reduce the dose of barbiturate required for induction of anesthesia (Greene, 1999). However, side effects including bradycardia, decreased myocardial contractility, respiratory depression, systemic and pulmonary hypertension followed by hypotension were reported after xylazine administration (Kästner, 2006; Uzun et al., 2006; Changmin et al., 2010)

Diazepam, a benzodiazepine derivative, is usually administered with ketamine/xylazine combinations to enhance muscle relaxation, produce calming, anxiolysis and provide an anesthetic sparing effect (Green et al., 1981)

Diazepam was chosen in this study to assess its ability to prolong the duration of anesthesia, to enhance muscle relaxation, to reduce the dose of thiopental and thus to reduce the incidence and severity of thiopental-induced cardiac abnormalities such as tachycardia. The aims of this study were 1) To investigate the effects on rabbits when administered one of the following agents/combinations diazepam (D), xylazine (X), thiopental (T) and combinations (DX, DT, XT & DXT); 2) To examine the efficacy of the superior anesthetic combination when used during surgery likely to be painful (vasectomy and embryo transfer).
MATERIALS AND METHODS

Growing and adult New Zealand White rabbits were obtained from the house of lab animals, the Faculty of Medicine, University of Assiut, and used in this study. Animals were subjected to a thorough clinical examination to ascertain that the animals were free from any cardiovascular or pulmonary disease. All animals had free access to a commercial diet and fresh water and the appropriate animal care protocol was applied throughout the experimental period.

In order to produce anesthesia with a wide safety margin, the dosage of thiopental, diazepam and xylazine used in this study were chosen so they do not exceed the suggested doses of previous studies (Sanford and Colby, 1980; Clifford, 1984; Sedgwick, 1986).

Experiment I:
A total of thirty two growing White New Zealand female rabbits of 1.0 kg average body weight were randomly allotted to eight groups, four animals each. The first group was injected with physiological saline. The other seven groups were injected with diazepam (Neuril 2.5 mg/kg; Memphis Co. for Pharm & Chemical Ind., Cairo, Egypt), xylazine (Xyla-ject 10 mg/kg; ADWIA Co. S. A. E. 10th of Ramadan city, Cairo, Egypt), thiopental (T 20 mg/kg) or their combinations (DX, DT, XT or DXT). Rectal temperature (RT), respiration (RR) and heart rates (HR) were recorded before and 10 min after injection. Induction of sedation and anesthesia was monitored by the animal’s reflexes in response to toe pinching.

Experiment II:
Eight adult White New Zealand rabbits (4 males and 4 females) of 2.0-3.0 kg average body weight were used in this experiment. Male rabbits were anesthetised using DXT established protocol (experiment 1). A scrotal incision (1-2 cm) was made to expose the vas deferens which was ligated by two ligatures (silk thread) and a segment (0.5 cm) was cut off between the two ligatures (Kong et al., 2004). After closing the skin, penicillin G. sodium (8 × 10⁶ units) was intramuscularly injected for five consecutive days. Vasectomised rabbits were given a period of 2 weeks to recover before using them as non fertilized bucks to stimulate and physiologically prepare the recipient females to receive embryos from other pregnant does throughout embryo transfer operation.

Induction of sedation and anesthesia:
Diazepam (2.5 mg/kg) and xylazine (10 mg/kg) were intramuscularly injected, whereas thiopental (20 mg/kg) was injected into marginal ear vein. The combination injections, diazepam and/or xylazine were given three minutes prior to thiopental administration. The induction and maintenance of sedation, analgesia and anesthesia was assessed according to the rabbit’s responsiveness to a painful stimulus (toe pinching). The degree of sedation was ranked to three levels based on the following criteria; 1) the animal was responsive to stimulus, able to stand and raise the head (first degree of sedation). 2) The animal was responsive to stimulus, occasionally moves the head and tends to stay in recumbent position (second degree of sedation). 3) The animal was partially responsive to stimulus and unable to move from recumbent position (third degree of sedation).

In the case of anesthesia, the time from drug administration till the animal starts gaining consciousness was classified into three periods; onset of anesthesia (period I), duration of anesthesia (period II) and sleeping time (period III). The onset of anesthesia is the moment when the animal loses consciousness and pain sensation. Duration of anesthesia extends from the onset of anesthesia until the animal starts being responsive to painful stimulus. Sleeping time starts when the animal is responsive to painful stimulus and ends when the animal starts moving.

Experimental measurements:
Rectal temperature was measured using a digital clinical thermometer inserted 4 cm into the rectum. The respiratory rate (RR) was measured by visually counting the chest flank movements for one minute using a stopwatch, when the animal was sitting quietly. The heart rate (HR) was obtained by counting the heartbeats for one minute using a stethoscope.

Statistical analysis:
Data were analyzed by ANOVA using the GLM procedure of SAS (SAS institute, 1999). When treatment effects were significant, differences between least squares means were tested using Duncan’s multiple-range test and the differences were considered significant at the level of P < 0.05.

RESULTS AND DISCUSSION

Effects of drug administration on rectal temperature, respiration and heart rates:
The effects of xylazine, diazepam, thiopental and their combinations on rectal temperature, respiration and heart rate are presented in Table 1. Administration of xylazine or diazepam decreased respiration and heart rates. Thiopental injection depressed respiration but increased heart rates. No significant changes were observed in rectal temperature following xylazine or diazepam injection. However, rabbits treated with thiopental exhibited higher rectal temperatures. A significant D × X × T interaction in respiratory and heart rates were observed. The lowest heart rate was observed in rabbits injected with diazepam alone followed by those treated with xylazine alone or in combination with diazepam and/or thiopental. Groups treated by thiopental alone or in combination with diazepam exhibited the highest heart rates. Separately or in combinations, injection of drugs resulted in decreased respiratory rates. The depression observed in respiration following xylazine injection was more

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pronounced compared to that noted after thiopental or diazepam administration (73 vs 105 and 110 breath/minute, respectively). The function of respiration was greatly influenced when rabbits were given the drugs in combinations rather than single injections.

A significant xylazine × thiopental interaction was observed in rectal temperature and respiration rate (see Table 1). Rabbits treated by thiopental without xylazine administration had higher rectal temperatures than their counterparts injected with xylazine or those not injected with both drugs. Although both drugs resulted in a decreased respiration rate, the depression noted in groups given xylazine with or without thiopental was more pronounced than that observed in groups injected with thiopental without xylazine administration.

Table 1: Rectal Temperature, respiration and pulse rates upon injection with, diazepam, xylazine, thiopental and their combination in rabbit

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rectal Temperature</th>
<th>P Value</th>
<th>Respiratory Rate</th>
<th>P Value</th>
<th>Pulse Rate</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>S</td>
<td>39.25</td>
<td>39.30</td>
<td>0.80</td>
<td>135</td>
<td>144</td>
<td>0.29</td>
</tr>
<tr>
<td>D</td>
<td>39.35</td>
<td>39.15</td>
<td>0.27</td>
<td>140</td>
<td>110***</td>
<td>0.001</td>
</tr>
<tr>
<td>X</td>
<td>39.20</td>
<td>39.95</td>
<td>0.14</td>
<td>137</td>
<td>73***</td>
<td>0.01</td>
</tr>
<tr>
<td>T</td>
<td>39.20</td>
<td>39.55*</td>
<td>0.02</td>
<td>135</td>
<td>105***</td>
<td>0.01</td>
</tr>
<tr>
<td>DX</td>
<td>39.20</td>
<td>38.97</td>
<td>0.47</td>
<td>132</td>
<td>52***</td>
<td>0.001</td>
</tr>
<tr>
<td>DT</td>
<td>39.25</td>
<td>39.62*</td>
<td>0.04</td>
<td>140</td>
<td>82***</td>
<td>0.01</td>
</tr>
<tr>
<td>XT</td>
<td>39.22</td>
<td>39.27</td>
<td>0.84</td>
<td>136</td>
<td>63***</td>
<td>0.001</td>
</tr>
<tr>
<td>DXT</td>
<td>39.20</td>
<td>39.27</td>
<td>0.76</td>
<td>134</td>
<td>40***</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Sedation and anesthesia:**

Sedation and anesthesia responses of rabbits differed upon injection with the aforementioned drugs. Although rabbits treated with X, D, T, DT and DX were all responsive to painful stimulus; they showed signs of different degrees of sedation. Rabbits injected with X or D were conscious and had first degree of sedation; while, those treated with T or DT exhibited signs of second degree of sedation. Injection of DX induced third degree of sedation as little withdrawal reflexes were observed in response to a painful stimulus. On the other hand, rabbits treated with XT or DXT combinations were unconscious and unresponsive to stimulus (complete analgesia and anesthesia). Interestingly, the duration of surgical anesthesia increased significantly from 15 ± 3.5 to 37 ± 2.9 min due to D inclusion to the XT combination.

**Experiment II:**

The use of DXT combination produced analgesia and anesthesia which was deep and long enough (37 mins) to accommodate surgical intervention. The duration required for performing surgical procedures averaged 30 min. Surgeries were conducted without serious complications. Decreases in respiration and heart rates were observed during time of anesthesia. However, they returned to baseline values within one hour and the recovery was smooth. All vasectomised males survived and used in preparation of recipient females. In addition, recipient females survived and gave progeny upon morula/balstocyst transfer to uterine horn.

**RESULTS AND DISCUSSION**

Results of the current study demonstrated that a combination of DXT can be used for induction of surgical anesthesia characterized by smooth recovery and of sufficient duration and depth for performing vasectomy and embryo transfer in rabbits without serious impairment of vital functions. However, transient negative side effects were observed following drug administration such as changed heart rate, hyperthermia and depressed respiration rate.

A significant body of evidence showed that xylazine causes decreased myocardial contractility, bradycardia and decreased cardiac output (Kul et al., 2000; Ismail et al., 2010). These cardiovascular side effects are alike in different species (Greene and Thurmon, 1988) Decreased heart rate could be attributed to sinus carotid baroreceptor reflex in response to an initial hypertension due to vasoconstriction caused by peripheral postsynaptic adrenoreceptors (Garner et al., 1971) Xylazine administration resulted in decreased heart rate, systolic and/or diastolic blood pressure in pony mares and heifers (Araujo and Ginther, 2009) pregnant cows (Hodgson et al., 2002) pregnant goats (Sakamoto et al., 1996) and in dogs (Ilbäck and Stalhandske, 2003).

The effects of benzodiazepine vary considerably among species (Rail, 1990). In general, benzodiazepines may cause minimal cardiovascular and moderate respiratory effects (Booth, 1988). In swine, increasing midazolam dosage, a benzodiazepine derivative drug, resulted in a progressive decrease in heart rate, but with maintaining cardiac output unchanged (Smith et al., 1991). Similarly, diazepam administration significantly decreased heart rate in preweaned harbour seal pups, particularly during the first 20 min post-administration, with a profound bradycardia being evident in two pups out of eighteen experimental animals (Lapiere et al., 2007).

In the current study, thiopental administration resulted in increased heart rates. This result is consistent with that observed in sheep where thiopental administration increased heart rate and arterial blood pressure by approximately 50 % (Runciman et al., 1997) Similarly, a 33% increase in heart rate with decreased cardiac output was observed in sheep injected with thiopental (Huang et al., 1997) The increase in heart rate was maintained from 0.5 min post-injection onwards (more than 30 min). The authors attributed these cardiovascular effects to enhancing sympathetic nerve stimulation caused by hypercarbia and acidosis, but not to baroreceptor
reflex because baroreflex can cause an initial increase in heart rate but can not sustain it throughout the experimental period. Pentobarbital administration also increased heart rates in dogs (Booth, 1988), and in rabbits when subanesthetic dose was administered (Murthy et al., 1982)

In this study, injection of all drugs was accompanied by a reduction in respiratory rate. The use of alpha 2-agonists as sedatives results in a decrease in respiratory rate for varying durations depending on the species and the dosage. This reduction occurs due to a CNS depression produced by alpha 2-adrenoreceptor stimulation (Lammintausta, 1991). A decrease in respiratory rate was noted following xylazine administration in rock partridges (Uzun et al., 2006) and cows (Vesal and Ahmadi, 1998). Contrary, xylazine appeared to have marginal effects on the respiratory system and blood gas values of rhesus monkeys (Reutlinger et al., 1980)

Although, benzodiazepine drugs were reported to have moderate depressant effects on respiratory system, considerable variations among species exist (Rail, 1990). In swine, midazolam resulted in a decreased respiratory rate. Diazepam led to a respiratory depression in rabbits when only used at a lethal dose (Bradshaw and Pleuvry, 1971); while, it did not significantly alter respiratory rate in partridges (Uzun et al., 2006). The reduction in respiratory rates occurred in the current study following diazepam injection can be attributed to its muscle relaxant property.

Barbiturates were reported to have potent respiratory depressant effects (Gaudy et al., 1983). Incidence of severe respiratory depression as a consequence of using pentobarbital was described (Flecknell et al., 1983; Peeters et al., 1988, and Borkowski et al., 1990). Similarly, thiopental administration decreased respiratory rate in sheep (Huang et al., 1997).

A significant body of evidence showed that benzodiazepines prolong barbiturate-induced anesthesia (sleeping time) in laboratory animals (Dobkin, 1961; Jori et al., 1969; Hester et al., 1971. and Chambers and Jefferson, 1977). The mechanism by which benzodiazepines and barbiturates synergistically interact is not fully understood. However, this supra additive hypnotic action can be attributed to the fact that both drugs interact with γ-aminobutyric acidA (GABA_A) receptor/chloride ionophore complex, the chief inhibitory neurotransmitter system in the brain (DeLorey et al., 1993). In conclusion, surgical anesthesia was induced when both xylazine and thiopental were administered in combination. Diazepam prolonged the duration of anesthesia when given with XT combination.

REFERENCES


