Effects of Tramadol on Liver and Renal Biochemistry and Histopathology in Dogs Undergoing Surgery under Pentobarbitone Anesthesia

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Abstract: Effect of single and repeated doses of tramadol on hepatic and renal functions and on pentobarbitone anesthesia was evaluated in dogs. Twenty five dogs were randomly distributed into five equal groups viz groups I and II received 3 mg kg⁻¹ of tramadol by subcutaneous and intravenous injections, respectively during premedication alone. Groups III and IV received similar doses of tramadol during premedication which was repeated 2 h after the initial dose through subcutaneous and intravenous injections, respectively. Group V served as negative control. Blood samples (2 mL) were taken at 0, 2 and 4 h after tramadol’s administration while liver and kidney biopsies were taken before the surgery ended. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels were significantly lowered in groups II-IV when compared to the group V at 4 h. Significant increase in AST was observed in groups I, III and V at 2 h and at 4 h in groups I and V. No significant change in kidney profile. Histopathological changes in the liver and kidney biopsies are mainly congestion, edema and cellular infiltration which occurred less frequently in groups III and IV. The volume of pentobarbitone was significantly lowered in groups III and IV. It is concluded that repeated administrations of tramadol at 3 mg kg⁻¹, IV or SC is safe at a frequency of 2 h interval during surgery without causing irreversible hepatic and renal damage and reduced the required dose of pentobarbitone needed to maintain anaesthesia in healthy dogs.

Key words: Tramadol, dog, liver, kidney, surgery, Malaysia

INTRODUCTION

There is an increasing concern in the control of acute and chronic pains in animals (Dekhordi et al., 2010; Wootton et al., 1988; Abu-Seida, 2012). Pain management is widely recognized as an essential component of clinical veterinary care in dogs and cats (Taylor, 2003). Currently, Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are the most widely used analgesics in dogs. Despite their easy availability and therapeutic advantages, NSAIDS’ side effects limit their use (Kukanich and Papich, 2004). Furthermore, they are ineffective in managing severe surgical pains (Kukanich and Papich, 2004). The application of transdermal patches is not a useful alternative because of the higher cost and potential hazards (Riviere and Papich, 2001). Hence, opioids remain the analgesics of choice in the management of moderate to severe surgical pains (Atici et al., 2005; Kongara et al., 2010; Mercadante and Arcuri, 2004).

Tramadol is an analgesic with a dual mechanism of action. It binds to the μ₁-opioid receptor (Guedes et al., 2005; Kukanich and Papich, 2004) as well as inhibits the monoaminergic pathway, i.e., the Noradrenaline (NA) and serotonin (5HT) reuptake pathways (Kubota et al., 2008; McMillan et al., 2008). Due to this reason, tramadol is also coined as an atypical opioid and is only partially inhibited by the opioid receptor antagonist naloxone (McMillan et al., 2008).

The strategy for administering opioids and other groups of analgesics before surgery is referred as preemptive analgesia which is aimed at preventing central sensitization of nociception (Mastrocinque and Fantoni, 2003). Preemptive administration of tramadol is found to significantly reduce the requirements of isoflurane among human patients (Wordliczek et al., 2002) and sevoflurane in dogs (Sekighi et al., 2009). Tramadol is metabolized by an isoenzyme Cytochrome P-450 2D6 (CYP2D6) (Kukanich and Papich, 2004) to produce an
active metabolite O-desmethyltramadol (M1). This is mainly mediated by the hepatic canine ortholog of CYP2D15 (Tasaki et al., 1998) before its rapid elimination by the kidneys (McMillan et al., 2008). Liver and kidneys are often predisposed to toxic injury due to their active role in drug metabolism and excretion. Both endogenous and exogenous opioids strongly influence renal functions through various mechanisms (Mercaudante and Arcuri, 2004) leading to various degrees of injury in chronic administration (Atici et al., 2005). Due to the fact that tramadol has a rapid elimination half-life, more frequent dosage intervals are needed to potentiate effective pain control such as that caused by major surgeries (Kukamich and Papich, 2004; McMillan et al., 2008). However, a potential risk in increased peroxidation, hepatic and renal damage due to long use of opioids was reported in rats (Atici et al., 2005).

In recent years, interest in studying renal and hepatic function during anesthesia and surgery in small animals has increased greatly (Kongara et al., 2009). Despite the increased use of tramadol in the management of chronic and postoperative pain in animals, there is limited information on its effects on hepatic and renal functions following its repeated administration in prolonged surgical procedures in dogs. Many previous studies focused on physiological changes that occur during the surgery (Wunsch et al., 2010) which can be attributed to immediate problems associated with cardiopulmonary functions in most surgical procedures (Valtolina et al., 2009; Wunsch et al., 2010). Thus, to incorporate an appropriate analgesic protocol, it is essential that both laboratory and clinical investigations are taken into consideration. More importantly, the differential effect of tramadol in dogs differs from other species (Vettorato et al., 2010) and their increased sensitivity to NSAIDs (Lascelles, 1999). Therefore, the objective of this study was to investigate the effects of the repeated intraoperative administration of tramadol on hepatic and renal blood biochemistry and pathological changes in dogs and to determine its effect on pentobarbital sodium’s requirement for maintenance of anesthesia.

MATERIALS AND METHODS

Animals: Twenty five mongrel dogs of both sexes weighing between 10 and 19 kg (12.8±2.5) and ageing approximately between 1 and 4 years old (2.3±0.8) were used in this study. They were allowed drinking water ad libitum until 2 h to the preanaesthetic medication while food was withheld 12 h before anaesthesia. The dogs were randomly assigned into five groups of equal number viz., groups that received either a single subcutaneous (group I) or single intravenous (group II) dose of tramadol (Unichem Laboratories Ltd. Ghaziabad India) at 3 mg kg⁻¹ during premedication. The other two remaining groups were given either a repeated subcutaneous (group III) or a repeated intravenous premedication (group IV) of a dose of 3 mg kg⁻¹ tramadol 2 h intra-operatively in addition to the premedication doses an hour before surgery. Group V without tramadol throughout the surgery acted as a negative control. Tramadol dosage was determined based on established data (Kukamich and Papich, 2004; McMillan et al., 2008). The dogs were physically examined by an experienced veterinary doctor before being included in the student surgery practice. This is a double blinded study where both the anaesthetist and surgeon were unaware about the treatment. Ethical approval for the study was granted by the University Animal Care Utility Committee of Universiti Putra Malaysia (UPM/FFV/PS/3.2.1.551/AUP-R86).

Experimental protocol

Anaesthesia and surgical protocols: Acepromazine maleate and atropine sulphate were administered subcutaneously to all dogs as a pre-anaesthetic agents at 0.1 and 0.04 mg kg⁻¹, respectively. General anesthesia was induced with pentobarbital sodium (30 mg kg⁻¹) via the cephalic vein using 21G butterfly catheter within approximately 30 min from the time of pre-anaesthesia. Pentobarbital sodium was administered until jaw relaxation was sufficient to allow tracheal intubation. Anaesthesia was maintained using pentobarbital sodium and the dogs were allowed to breath spontaneously in room air. Total volume of pentobarbital sodium for induction and maintenance of anaesthesia, body weight of dogs, duration of anesthesia and surgery were recorded.

Blood sampling and biopsy: Approximately, 2 mL of blood sample was collected from each dog into plain tube at baseline via a lateral saphenous vein. Subsequently, tramadol was administered followed by blood collection at two different time intervals (2 and 4 h) via jugular venipuncture to avoid interruption with the surgery (exploratory laparotomy and ovariohysterectomy). All samples were collected into 3 mL plain tubes and the blood was allowed to clot at room temperature before being centrifuged immediately (1000 g for 10 min) to separate the serum.

A biopsy sample from the hepatic margin was obtained by guillotine method (Theresa, 2007) at the end of surgery. Briefly, a loop of suture was placed at approximately 2 cm from the protruding margin of the liver lobe and the ligature was pulled tight to crush the hepatic parenchyma. The blood vessels and biliary ducts were properly ligated before a cut at an approximately 5 mm distal to the ligature was conducted. Using a No. 15 scalpel blade, a wedge biopsy of the kidney was obtained.
by making two incisions into the renal parenchyma at an angle (approximately 30°) perpendicular to each other. The incision was closed in a mattress suture pattern using a 3-0 absorbable suture material. Finally, liver and kidney biopsies were immediately fixed in a 10% formalin for histopathology until further processing.

**Serum chemistry analysis:** Serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LDH), concentration of Blood Urea Nitrogen (BUN) and serum creatinine levels were measured using an automatic biochemistry analyzer (Hitachi 902 automatic chemistry analyzer, United State) by kinetic UV assay, rate-blanked and compensated, urea/urea nitrogen and creatinine Jaffe method (Roche diagnostics GmbH, Mannheim USA) according to the manufacturer’s instructions (Atici et al., 2005).

**Histopathologic examination:** Following fixation, liver and kidney biopsies were automatically dehydrated in ascending grade of alcohol (ethanol), cleared in xylene (BDH Laboratory Pode, England) and embedded in paraffin wax (BDH Laboratory Pode, England). Samples were blocked with paraffin wax and then sectioned at 4 μm using a microtome (Leica 2045, Germany). Following this the sections were mounted on glass slides and stained with hematoxylin (Sigma Eldrich, Germany) and eosin (Sigma Eldrich, Germany). The slides were dipped in xylene and the cover slip was slipped. Examination of the slides was carried out using a light microscope (Olympus BX51, Japan) which was coupled with an image analyzer (Computer Analysis LS Research).

**Statistical analysis:** Total volume of pentobarbitone sodium for induction and maintenance of anesthesia, body weight of dogs, duration of anesthesia and surgery were compared using one-way ANOVA. Serum chemistry parameters were compared across time and treatment groups using a two-way ANOVA test. All parameters were expressed as means±SD while histopathological changes were evaluated in terms of disease severity. Significant different means were examined using the Duncan multiple range test. All statistical analysis were conducted at 95% confidence level using SPSS Version 16 (SPSS Inc. Chicago USA).

**RESULTS AND DISCUSSION**

**Anesthesia and surgery record:** Mean duration of surgery duration of anesthesia and body weight of the dogs were not significantly different within and between the groups. However, the mean volume of pentobarbitone sodium was significantly lowered in groups III and IV compare to groups I, II and V (Fig. 1a-d).

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Fig. 1a-d: SSC Single Subcutaneous; SIV Single Intravenous; DSC repeated subcutaneous; DIV repeated intravenous; CNTRL Negative Control. **means with superscripts differ significantly at p<0.05 between groups
Table 1: Liver and kidney serum profiles of dogs at baseline and after tramadol administration in the different treatment groups

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>BUN (mg/dL)</th>
<th>Creatinin (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>25.84±5.1600</td>
<td>33.92±4.9200</td>
<td>7.30±1.02</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>28.86±11.4000</td>
<td>46.58±4.8200</td>
<td>7.02±1.41</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>45.50±11.4300</td>
<td>53.54±17.5500</td>
<td>6.88±1.30</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>23.42±10.1100</td>
<td>30.12±4.2100</td>
<td>5.90±1.74</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>23.78±11.9900</td>
<td>24.98±4.9000</td>
<td>5.76±1.88</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>22.52±10.1800</td>
<td>26.39±4.6600</td>
<td>5.64±1.78</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>30.00±16.3200</td>
<td>31.90±4.0800</td>
<td>5.02±1.55</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30.42±16.3400</td>
<td>41.44±5.8000</td>
<td>5.78±1.60</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>29.96±16.1400</td>
<td>41.98±8.3200</td>
<td>5.30±1.44</td>
</tr>
</tbody>
</table>

ALT = Alanine Transaminase; AST = Aspartate Transaminase; BUN = Blood Urea Nitrogen; group I single subcutaneous; group II single intravenous; group III repeated subcutaneous; group IV repeated intravenous; group V negative control; * means within columns with superscripts differ significantly at p<0.05 between groups.

Table 2: Histopathological changes in the liver among the various treated groups (n = 5/group)

<table>
<thead>
<tr>
<th>Lesions</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular infiltration</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Edema</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Congestion</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Vacuolization</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Neerosis of hepatocytes</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Group I single subcutaneous; group II single intravenous; group III repeated subcutaneous; group IV repeated intravenous; group V negative control. The numbers in the table indicate number of dogs affected in each group.

Serum chemistry analysis: At 4 h, serum ALT and AST were not significantly affected among the experimental groups (II-IV) when compared group V. In addition to a significant increase in serum AST level at 2 h of blood collection among groups I, III and V, a significant increase was also observed at 4 h among groups I and V (Table 1). However, no significant increase was found in BUN and serum creatinine levels in all of the groups throughout the sampling period.

Histopathologic examination: Examining the liver, all the groups showed cellular infiltration and congestion ranging from mild (groups III and IV), moderate (groups I and II) to severe (groups V) (Fig. 2). In addition, necrosis of the hepatocytes and diffused vacuolation of the hepatocyte were also observed in the group V (Table 2).

In the kidneys, cellular infiltration and tubular edema were the major histopathologic changes observed for all of the groups. Again, these changes were less severe in the groups III and IV (Fig. 3). Additionally, congestion, interstitial edema and focal tubular and glomerular necrosis were seen mostly among groups I, II and V when compared to groups III and IV (Table 3). Despite the long time use of opioids in dogs, little is known on the effects of tramadol in dogs undergoing a long surgical procedure.

The primary role of liver and kidney in drug metabolism and excretion predispose these organs to toxic injury. Tramadol does increase serum ALT, AST and LDH in line with what is expected of opioids. Hence, the ability to cause hepatotoxicity and nephrotoxicity have been reported during opioids metabolism and excretion (Atici et al., 2005). In this study, ALT and AST were found to be lowered and remained within the normal range among the treatment groups (II-IV) at 4 h post tramadol.
creatinine levels remained unaffected throughout the experiment within the tramadol treated groups indicating that tramadol does not affect the renal function and is safe when administered repeatedly during a prolonged surgery at the recommended dose. These results verify further the notions that therapeutic doses of opioids administration are unlikely to cause irreversible changes (Gomez-Lechon et al., 1987). In addition, the histopathological changes observed in the liver and kidney biopsies were due to decreased cardiac output effect of pentobarbitone sodium, hence decreased renal blood perfusion (Wada et al., 1996). Barbiturate is reported to cause hypersensitivity reactions and biliary congestion without causing irreversible injurious effect on the hepatic function (Sato et al., 1989).

An evidence of renal damage such as tubular vacuolation, mononuclear cell infiltration, focal necrosis and haemorrhage in addition to increased serum BUN and creatinine levels in rats receiving long-term morphine administration was reported (Atici et al., 2005). Renal damage was also reported in experimental rats placed on a long-term administration of morphine-like agent LAAM (Almeida et al., 2010). On the other hand, serum BUN and creatinine levels remain unchanged among rats receiving long-term tramadol (Atici et al., 2005) and in sheep five days after tramadol administration (Dehkordi et al., 2010). However, minimal histological changes confined to the tubular cells were observed in rats receiving long-term tramadol administration (Atici et al., 2005).

No significant differences was observed throughout the groups with respect to body weight distribution, duration of anaesthesia and the duration of surgery. However, there was a significantly lowered volume of pentobarbitone sodium needed to maintain anaesthesia in groups (III and IV) receiving additional doses of tramadol. This is could due to the fact that tramadol acted synergistically with pentobarbitone sodium and complemented its action hence lowering the amount of anaesthetic drug required to maintain surgical plane anaesthesia as reported by Seddighi et al. (2009) and Wordliczek et al. (2002) with other anaesthetic agents.

CONCLUSION

Researchers conclude that repeated administrations of tramadol at 3 mg kg\(^{-1}\), IV or SC is safe at a frequency of 2 h interval during a prolong surgery without causing irreversible hepatic and renal damage in healthy dogs. Similarly, repeated administrations of tramadol intraoperatively enhance analgesia thereby reducing the required dose of pentobarbitone sodium needed to maintain anaesthesia during a prolonged surgery.
REFERENCES


