

Single versus repeated tramadol injection in laparotomized albino rats: comparison of effects on hematology, serum biochemical parameters, and body weight gain

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ABSTRACT

This study was aimed to assess the effects of single and repeated tramadol administration on some hematological and serum biochemical parameters of laparotomized rats. Laparotomized rats (n=18) were randomly divided into three equal groups. Normal saline was administered to the rats of group 1 (control). Tramadol (dosed at 10 mg/kg bwt) was administered singly to the rats of group 2. The same dose of tramadol was administered repeatedly every 12 h up to 72 h to the rats of group 3. On day 3 and 10 post-laparotomy (PSL), blood urea nitrogen, serum creatinine, total serum protein, hematocrit, hemoglobin concentration and red blood cell counts in the rats of group 2 were found to be significantly ($P<0.05$) higher than those obtained in group 1 and group 3. Mean weights of rats in group 1 and group 3 were significantly lower than those of the rats of group 2 PSL. This study showed that repeated tramadol administration lead to slower weight gain as well as marked decrease in biochemical and erythrocytic variables in rats. Therefore, single administration of tramadol PSL may suffice for analgesia.

Keywords

Tramadol hydrochloride, Laparotomy, Opioid, Erythrocytic indices

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INTRODUCTION

The use of analgesics to prevent or treat post-operative pain in rodents has been encouraged as a refinement

method (Guzman-Silva et al., 2007). In rodents as well as other animals, the choice of analgesic is influenced by the degree of pain that is actually present (Flecknell, 1994). Opioids are usually preferred drugs for the management of severe and chronic pain (Kaye et al., 2010). They decrease perception and reaction to pain as well as increase pain tolerance (Jablonski et al., 2000). Their use for analgesia presents undesirable effects such as respiratory depression, cardiovascular depression, sedation and constipation (Quang-Cantagrel et al., 2000). Therefore, in clinical veterinary practice, proper evaluation of potential benefits of an analgesic treatment regimen for a particular procedure is very imperative. This is because, inappropriate use of a potent analgesic predisposes to undesirable side effects of the agent which may outweigh any potential pain alleviating effects (Flecknell, 1994).

Tramadol hydrochloride is a centrally acting synthetic 4-phenylpiperidine analogue of codeine used in the treatment of moderate to severe pain (Lee et al., 1993; Scott and Perry, 2000). It acts as an opioid agonist with selectivity for the mu opioid receptor and binds weakly to the kappa and delta opioid receptors (Bamigbade and Langford, 1998). Tramadol is extensively metabolised in the liver, and the *O*-desmethyl metabolite displays a 200-fold higher affinity for opioid receptors than the parent drug (Lee et al., 1993). Tramadol's analgesic effects have been associated with the serotogenic system through the inhibition of the serotonin function (Habibian-Dehkordi et al., 2010). The efficacy of tramadol in pain relief in small animal medicine has been confirmed in previous studies (Vettorato et al., 2010; Cagnardi et al., 2011). This drug has a wide safety margin and unlike other opioids has minimal cardiovascular and respiratory side effects (Quang-Cantagrel et al., 2000). Studies have

demonstrated that short term intravenous administration of tramadol had no effect on hepatic enzyme levels of dogs (Akhtardanesh et al., 2014) and rabbits (Udegbunam et al., 2014). Also, no significant change in blood urea nitrogen (BUN) and creatinine was recorded after short term (Akhtardanesh et al., 2014; Udegbunam et al., 2014) and chronic use of tramadol (Atici et al., 2005).

Despite the constant use of tramadol in animals, there is limited data on the requisite analgesic dosage of tramadol for the management of laparotomy-induced abdominal pain in rats. Also, no work has established the effect of short term tramadol's use on the hematology of laparotomized rats. In this study, we compared the effect of single and repeated use of tramadol on some hematological and serum biochemical parameters as well as body weight gain in laparotomized rats.

MATERIALS AND METHOD

Animals: Wistar rats (n=18) weighing between 140-164 g were used for this study. They were fed commercial breeders chow (Vital feeds, Jos). Clean water was provided *ad libitum*. After 2 weeks of acclimatization, the rats were assigned to three equal groups. The protocols used for this research were approved by the Animal Ethics Committee, University of Nigeria, Nsukka (approval no. UNAEC/15/382).

Methodology: Three groups of rats (n=6/group) underwent exploratory laparotomy under deep pentobarbitone anesthesia (dosed at 35 mg/kg bwt, intraperitoneally). Immediately after surgery, single dose (dosed at 10 mg/kg bwt) tramadol (Tramal®, Guantal, Germany) was administered intramuscularly to rats in groups 2. Tramadol (dosed at 10 mg/kg bwt) was administered intramuscularly at the end of surgery to rats in group 3 and subsequently every 12 h for 72 h. The control rats (group 1) were injected with normal saline every 12 h for 72 h. The effects of tramadol on rats were assessed on day 1, 3 and 10 post-laparotomy (PSL) as shown below:

Serum biochemical assay: On day 1, 3 and 10 PSL, blood samples (3 mL) were collected from retro orbital plexus of rats. Blood on collection were dispensed into sample bottles without anticoagulants. Serum were separated from clotted samples within 30 min of blood collection. BUN was assayed as described by Fawcett and Scott (1960), serum creatinine determination was done using the modified Jaffe method (Blass et al., 1974) while total serum protein was determined by direct biuret method (Lubran, 1974).

Hematology: Also on day 1, 3 and 10 PSL, blood samples (1 mL) were collected from retro orbital plexus of rats into sample bottles containing ethylene diamine tetra acetate (EDTA). Hematocrit (HCT), hemoglobin concentrations (HBC), red blood cell (RBC) counts and white blood cell (WBC) counts were determined as described by Bain et al. (2012).

Weight determination: Animals in each group were weighed using digital weighing balance and their weights recorded before surgery and subsequently on day 1, 3, 6, 8 and 10 PSL.

Data analysis: Data obtained were summarized as mean (standard error of means). To determine the effect of the treatments, mean data on hematology, serum biochemical assay and weights were compared between groups using one-way analysis of variance followed by Duncan multiple range test. Probability <0.05 were considered as significant.

RESULTS AND DISCUSSION

The results of the biochemical parameters presented in **Table 1** shows that mean BUN, creatinine and total serum protein of group 2 were significantly ($P<0.05$) higher than those obtained in groups 1 and 3 on day 3 and 10 PSL. The mean values of these parameters were significantly ($P<0.05$) least in group 3 on day 10. Surgical stress leads to acute compensatory responses such as alteration in physiologic, neuroendocrine and metabolic status of animals (Desborough, 2000). These changes occur in the body's attempt to maintain homeostasis as well as hasten the rate of catabolism (Desborough, 2000; Lobo et al., 2013). In the immediate post-operative period (catabolic phase) which is marked by zero feed intake, rapid lysis of skeletal muscles leads negative nitrogen balance as amino acids are deaminated (Breznock, 1980). This leads to marked increase in BUN and creatinine post-surgery (Mohammad et al., 2008; Olaifa and Opara, 2011). Also BUN rises when their endogenous production from skeletal muscle catabolism outweighs the rate of urinary urea excretion or if glomerular filtration rate decreases (Orloff and Hutchin, 1972; Schaer, 1982; Lobo et al., 2013). In this study, the rats in all the groups consumed some feed from day 1 post-surgery. This might have prevented the rapid lysis of their skeletal muscle thus BUN of these rats did not rise. Also the decrease in BUN and creatinine recorded in the groups of rats might suggest that the glomerular filtration of rats in these rats increased post-surgery.

Table 1: Blood urea nitrogen, serum creatinine and total plasma protein of rats before and post laparotomy.

Parameters	Baseline	Post laparotomy		
		Day 1	Day 3	Day 10
Blood urea nitrogen (mg/dL)				
Group 1	23.0±1.58	11.0±0.77	10.6±0.73 ^a	13.1±0.93 ^a
Group 2	22.0±1.77	12.2±1.59	18.9±2.33 ^b	17.2±2.98 ^b
Group 3	22.8±2.30	14.8±1.5	10.0±1.4 ^a	7.99±0.80 ^c
Creatinine (mg/dL)				
Group 1	0.8±0.12	0.39±0.05	0.4±0.06 ^a	0.44±0.08 ^a
Group 2	0.87±0.24	0.47±0.06	0.97±0.04 ^b	0.77±0.07 ^b
Group 3	0.67±0.03	0.44±0.03	0.47±0.03 ^a	0.25±0.02 ^c
Total serum protein (g/dL)				
Group 1	6.60±0.59	5.50±1.20	4.60±0.14 ^a	5.90±0.43 ^a
Group 2	6.80±0.23	5.28±0.17	5.90±0.70 ^b	6.30±0.30 ^b
Group 3	6.70±0.60	5.67±1.00	4.00±0.18 ^c	5.60±0.43 ^a

Different superscripts^{a,b,c} in a column indicate significant difference between group means ($P<0.05$).

Table 2: Hematocrit (HCT), red blood cell (RBC) counts and hemoglobin (Hb) concentrations of rats before and post laparotomy.

Parameters	Baseline	Post laparotomy		
		Day 1	Day 3	Day 10
HCT (%)				
Group 1	36.40±0.70	34.00±1.30	26.00±1.20 ^a	29.90±1.20 ^a
Group 2	37.70±0.30	30.30±0.30	35.00±2.30 ^b	40.30±1.20 ^b
Group 3	38.30±1.90	31.00±1.20	27.70±2.40 ^a	37.00±1.20 ^c
RBC (10⁶/μL)				
Group 1	7.20±0.30	6.50±0.20	4.60±0.40 ^a	5.50±0.40 ^a
Group 2	7.00±0.12	5.00±0.10	6.20±0.20 ^b	6.70±0.17 ^b
Group 3	6.9±0.24	5.60±5.70	4.97±0.20 ^a	6.30±0.07 ^c
Hb (g/dL)				
Group 1	12.80±0.31	11.20±0.81	8.30±0.47 ^a	9.77±0.49 ^a
Group 2	12.27±0.41	9.90±0.29	10.87±0.09 ^b	11.47±0.27 ^b
Group 3	12.70±0.18	9.12±0.14	8.10±0.47 ^a	10.70±0.12 ^c

Different superscripts^{a,b,c} in a column indicate significant difference between group means ($P<0.05$).

Table 3: White blood cell counts ($\times 10^3/\mu\text{L}$) of rats before and post laparotomy.

Groups	Baseline	Post laparotomy		
		Day 1	Day 3	Day 10
Group 1	11200±378	12300±458 ^a	11900±425	11400±290
Group 2	10900±353	10700±251 ^b	11430±821	11650±463
Group 3	11600±529	11500±493 ^{ab}	11100±251	11800±144

Different superscripts^{a,b,c} in a column indicate significant difference between group means ($P<0.05$).

Table 4: Weights (g) of rats before and post laparotomy.

Groups	Baseline	Post laparotomy				
		Day 1	Day 3	Day 6	Day 8	Day 10
Group 1	154.00±12.50	149.30±12.30	147.0±13.10 ^a	146.30±3.80 ^a	151.30±2.60 ^a	157.00±8.50 ^a
Group 2	157.70±11.80	153.30±12.40	156.70±10.70 ^b	164.70±11.40 ^b	168.30±11.00 ^b	170.00±9.50 ^b
Group 3	157.0±13.30	143.70±12.00	140.70±8.90 ^a	146.00±7.60 ^a	149.00±7.70 ^a	165.30±3.70 ^c

Different superscripts^{a,b,c} in a column indicate significant difference between group means ($P<0.05$).

On day 3 and 10 PSL, mean HCT, Hb and RBC of rats in group 2 were significantly ($P>0.05$) higher than mean values of these parameters obtained in groups 1 and 3 (Table 2). Also on day 10 PSL, mean HCT, HbC and RBC of rats in group 3 were significantly ($P<0.05$) higher than those obtained in group 1. As shown in Table 3, WBC count of group 1 was significantly

($P<0.05$) higher than those of group 2 and 3 on day 1 PSL. Previous studies have shown that changes in hematological parameters post-surgery may provide a clue to the health status and recovery rate of animals (Mohammad et al., 2008; Olaifa and Opara, 2011). Based on the result of this study, we attribute the marked decrease in the studied erythrocytic variables

in all the groups on PSL 1 to the side effect of surgery. Post trauma, increased anti-diuretic hormone and aldosterone secretion with consequent water and salt retention causes the expansion of the extracellular fluid leading to hemodilution and drop in HCT (Rassam and Counsell., 2005; Lobo et al., 2013). In a similar study, these erythrocytic indices decreased after intravenous tramadol injection in sheep (Habibian-Dehkordi et al., 2010). The drop in RBC and HCT of rats in the group that received tramadol repeatedly might be the consequence of surgery, reduced feed intake and repeated tramadol use. The significantly higher WBC in the control group suggests that more stress and pain was felt by rats in this group since they were not given tramadol post-surgery. According to Olaifa and Opara (2011), WBC increases post-surgery as result of inflammation and stress associated with surgery.

On day 3, 6, 8 and 10 PSL, mean weights of rats in groups 1 and 3 were significantly lower compared to those of rats in group 2 (Table 4). Post-surgery, weights of the control group and those of the group repeatedly dosed with tramadol decreased up to day 6. Various studies have reported reduction in body weight and loss of appetite in rodents post operatively (Jablonski et al., 2002; Liles and Flecknell, 1992). It have been demonstrated that opioid analgesics such as nalbuphine, buprenorphine and butorphanol reduced food intake in intact rats (Liles and Flecknell, 1994). Furthermore, Jablonski et al. (2002) and Jacobson et al. (2000) reported weight loss in rats treated with buprenorphine post-surgery. Due to this effect of opioids on feed intake, their unnecessary administration can be detrimental to rodents (Flecknell, 1994; Jablonski et al., 2000). In this study continued weight loss in the group of rats dosed repeatedly with tramadol suggests reduced feed intake in the group of rats hence the consequent weight loss observed in them. An in vitro study of the effect of (+)-tramadol, (-)-tramadol and the major metabolite (O-desmethyl tramadol) on intestinal motility showed that O-desmethyltramadol inhibited peristalsis in guinea pig small intestine more than the parent compounds (Herbert et al., 2007).

CONCLUSION

Repeated administration of tramadol for up to 72 h post laparotomy was deleterious on the laparotomized rats. This manifested slower weight gain post-surgery as well as markedly decreases in the erythrocytic variables in rats. Thus, single injection of tramadol post laparotomy should suffice for analgesia in rats.

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