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Investigation of the analgesic and systemic effects of ibuprofen in rabbits

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ABSTRACT

Ibuprofen suspensions (15 and 30 mg/kg b.m.) were orally administered to two groups of rabbits every 12 hours for three days following laparotomy. The effect of these doses on the pain scores, haematology parameters, several serum enzyme levels and gastrointestinal tract were evaluated. The results were then compared with the untreated group but laparotomised. The administration of 30 mg/kg b.m. ibuprofen produced a significantly ($P<0.05$) lower pain score by 2 hours post administration. By 18 hours, the pain score of the group II rabbits (15 mg/kg) were significantly ($P<0.05$) lower than those of control (group I). However from 24 hours, group III (30 mg/kg) and group II (15 mg/kg) pain scores were not significantly ($P>0.05$) different. Administration of the two doses of ibuprofen did not significantly ($P>0.05$) alter the packed cell volume (PCV), red blood cell count (RBC) and haemoglobin concentrations (Hbc) of the rabbits. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) significantly ($P<0.05$) increased in the ibuprofen treated groups. Also a significant ($P<0.05$) increase in serum creatinine level was observed following the administration of 30 mg/kg ibuprofen. No melena, vomiting or diarrhoea were observed in rabbits in the control and treated groups. In conclusion, the two doses of ibuprofen were able to relief laparotomy pain at the dosing regimen used. However, the use of 15 mg/kg b.m. ibuprofen should be favoured since less renal side effects were noted following its use.

Key words: ibuprofen, rabbits, laparotomy, analgesia, haematology, serum enzymes

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are drugs that produce analgesia by peripheral effect (VANE, 1971; AITKENHEAD and SMITH, 1996). They reduce the activity of cyclo-oxygenase thus inhibiting the synthesis and release of prostaglandins

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and leukotrienes which are prime mediators of inflammation (GRISNEAUX et al., 1999; RAMPRABHU et al., 2001). These drugs are thus used in man and animals as antipyretics, anti-inflammatory agents and analgesics (FLOWER et al., 1985).

The inhibition of prostaglandin synthesis may lead to gastric and duodenal ulcers, renal failure and haemorrhage (GRISNEAUX et al., 1999). In addition blood thinning, hepatitis and anaemia has been reported following use of NSAID (KATZUNG and FURST, 1998; RAMPRABHU et al., 2001).

These drugs are commonly used for the treatment of rheumatoid and chronic musculoskeletal pains (AITKENHEAD and SMITH, 1996). However acetylsalicylic acid, carprofen, ibuprofen and acetaminophen have been found effective in the relief of post-operative pain in man and dogs (COOPER et al., 1989; LASCELLES et al., 1994; SCHOU et al., 1998).

Ibuprofen can be administered orally to rabbits (LILES and FLECKNELL, 1992). The drug is however, not currently recommended for the relief of post-operative laparotomy pain in this species. This study thus investigates the analgesic efficacy of ibuprofen for the relief of post-operative laparotomy pain in rabbits. Also some possible adverse effects of the doses used were investigated.

Materials and methods

Animals. Eighteen clinically healthy adult rabbits of both sexes were used for the study. Their mean (sem) body mass was 1.1 ± 0.5 kg. They were confined in metal cages. Pelleted feed (Vital feed; Jos) and water were provided *ad libitum*. The rabbits were kept for two weeks to stabilize before the work. Before the study, each rabbit was identified using picric acid. They were then randomly separated into 3 groups of 6 each.

Pre-experimental evaluation. The appetite, activity and posture of the rabbits were assessed to be normal before the experiment. The heart rate, respiratory rate and temperature of the rabbits were determined and recorded 10 minutes before the surgery. Blood was collected from the jugular veins of rabbits and dispensed into ethylene diamine tetra acetate coated and plain bottles. Whole blood was analysed for packed cell volume (PCV), red blood cell count (RBC) and haemoglobin concentration (Hbc) as described by SIMPSON (1996). Samples without anticoagulant were allowed to clot. The serum samples were then separated and centrifuged. The clear sera thus obtained were used for the determination of serum alanine aminotransferase (ALT) using the Reitman - Franknel colorimeteric method (REITMAN and FRANKNEL, 1957). Serum creatinine was also determined by the modified Jaffe method (BLASS et al., 1974). These were recorded as baseline values.

Anaesthetic protocol. Atropine sulphate (0.05 mg/kg b.m., Hamexmedica, UK) and acepromazine (0.5 mg/kg b.m., Vetoquinol SA, France) were administered intramuscularly

for pre-medication. Pentobarbitone (15 mg/kg b.m., Kyron Laboratories, Benrose) was then administered i.p. 10 minutes later to induce anaesthesia.

Surgical procedure. Following aseptic preparation of the midventral area of the rabbits, a 3 cm midline laparotomy incision was made. The intestine and stomach were exteriorized and then pushed back. The incision was then closed (KNECHTH et al., 1987).

Postsurgical care. The incision site was cleaned with methylated spirit. Procaine penicillin (6 mg/kg b.m., Helm pharmaceutical, Germany) and streptomycin (10 mg/kg b.m., Jopan pharmaceutical, Nigeria) were administered i.m. for 5 days. Immediately after recovery, 1 mL of normal saline was given per os (p.o.) to group 1 (control) rabbits. Subsequent administration of normal saline was done every 12 hours for 3 days. Group II animals were orally given ibuprofen suspension (Orbifen®, Orbis consumer Ltd., Northfields) 15 mg/kg b.m. immediately after recovery and then every 12 hours for 3 days. Group III rabbits were given ibuprofen suspension 30 mg/kg b.m. at the same dosing regimen as group II.

Assessment of pain. Objective and subjective pain assessments were made at 2, 18, 24, 42, 48, 66, and 72 hours during ibuprofen administration as follows.

Objective pain assessment. The heart rate (beats/minutes), respiratory rate (breaths/minute) and temperature (^{0}C) were measured as described by EZE and NWEKE (2004). These were expressed as percentage changes from their baseline values. These percentage changes were then used for objective pain scoring as described by GELLASCH et al. (2002) as follows: Percentage change less than 10% was scored as 0 while percentage change less than 20% was scored as 1. Percentage change less than 30% was scored as 2 while percentage change greater than 30% was scored as 3.

Subjective pain assessment. This was done by observing and scoring the following.

Activity: Normal activity was scored O, slightly less active as 1 and less active than normal was scored 2.

Posture: Normal posture was scored 0 while guarding/hunching was scored 1.

Appetite: Eating was scored 0 while not eating was scored 1. The total pain score per animal was the sum of both objective and subjective scores.

Investigation of systemic effects. On day 3, blood was also collected for the determination of PCV, RBC, Hbc, ALT, AST and creatinine.

Gastrointestinal tract (GIT) effects. The presence of melena, vomiting, and diarrhoea were looked for in the experimental rabbits.

Data analysis. The mean (SD) haematologic and serum enzyme values were compared using one-way analysis of variance (ANOVA). The mean pain scores were compared

using Kruskall - Wallis test. Tamhame and least significance difference (LSD) were used as post-hoc tests at P values less than 0.05.

Results

Pain score. The mean pain score was significantly (P<0.05) less in group 3 by 2 hours post ibuprofen administration. No significant (P>0.05) difference in pain score was noted between groups I and II at this time. By 18 hours, the pain score of group II became significantly (P<0.05) lower compared to that of group I. However between 24-72 hours, no significant (P>0.05) difference was noticed between the pain scores of groups 2 and 3 (Table 1)

Haematology. No significant (P>0.05) difference was observed in the mean PCV, RBC and Hbc among the 3 groups. (Figs. 1, 2, 3)

Serum assay. Administration of ibuprofen 15 mg/kg and 30 mg/kg b.m. lead to a significant (P<0.05) increase in both serum ALT and AST levels (Tables 2 and 3). Serum creatinine significantly (P<0.05) increased following administration of ibuprofen 30 mg/kg b.m. (Table 4)

GIT effect. No melena, vomiting or diarrhoea was observed in the experimental rabbits during drug administration.

Table 1. Pain scores (mean ± SD) of rabbits treated with ibuprofen 15 mg/kg b.m. and 30 mg/kg b.m.

Time (hours)	Pain scores		
	Group I	Group II	Group III
2	5.00 ± 0.00^{a}	5.17 ± 0.98^{a}	3.3 ± 0.82^{b}
18	4.80 ± 0.45^{a}	2.83 ± 0.75^{b}	0.67 ± 0.52^{c}
24	6.60 ± 0.55^{a}	2.00 ± 0.00^{b}	2.00 ± 0.89^{b}
42	5.80 ± 1.10^{a}	1.50 ± 0.55^{b}	1.50 ± 0.84^{b}
48	6.00 ± 0.71^{a}	1.50 ± 0.55^{b}	1.50 ± 0.84^{b}
66	6.00 ± 0.71^{a}	1.00 ± 0.00^{b}	0.83 ± 0.75^{b}
72	5.80 ± 1.10^{a}	1.00 ± 0.00^{b}	0.80 ± 0.75^{b}

^{abc} Different superscripts in a row indicate significant difference between the means of the groups at the probability level: P< 0.05. Group I: control., Group II: ibuprofen 15 mg/kg b.m., Group III: 30 mg/kg b.m.

Table 2. Serum ALT (mean ± SD) levels of rabbits treated with ibuprofen 15 mg/kg b.m. and 30 mg/kg b.m.

Groups	Serum ALT	
	Day 0	Day 3
I	28.91 ± 4.92	25.44 ± 3.99[a]
II	27.42 ± 5.14	40.66 ± 4.22[b]
III	29.11 ± 8.17	49.42 ± 7.15[c]

[abc] Different superscripts in a column indicate significant difference between the means of the groups at the probability level: P<0.05. Group I: control., Group II: ibuprofen 15 mg/kg b.m., Group III: 30 mg/kg b.m.

Table 3. Serum AST (mean ± SD) levels of rabbits treated with ibuprofen 15 mg/kg b.m. and 30 mg/kg b.m.

Groups	Serum AST	
	Day 0	Day 3
I	23.98 ± 3.90	22.09 ± 4.24[a]
II	24.38 ± 3.87	36.45 ± 6.14[b]
III	24.20 ± 5.06	37.61 ± 8.9b[b]

[ab] Different superscripts in a column indicate significant difference between the means of the groups at the probability level: P<0.05. Group I: control., Group II: ibuprofen 15 mg/kg b.m., Group III: 30 mg/kg b.m.

Table 4. Serum creatinine (mean ± SD) levels of rabbits treated with ibuprofen 15 mg/kg b.m. and 30 mg/kg b.m.

Groups	Serum creatinine	
	Day 0	Day 3
I	44.17 ± 15.53	39.76 ± 18.48[a]
II	43.56 ± 21.69	42.09[a] ±13.04[a]
III	49.99 ± 20.11	66.03[b] ± 15.35[a]

[ab] Different superscripts in a column indicate significant difference between the means of the groups at the probability level: P<0.05. Group I: control., Group II: ibuprofen 15 mg/kg b.m., Group III: 30 mg/kg b.m.

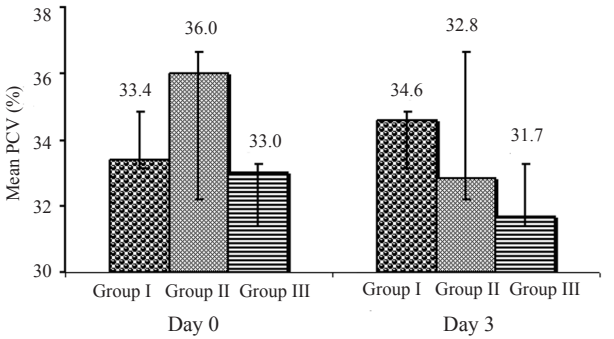

Fig. 1. The packed cell volume of rabbits treated with normal saline/control (Group I), 15 mg/kg ibuprofen (Group II) and 30 mg/kg ibuprofen (Group III)

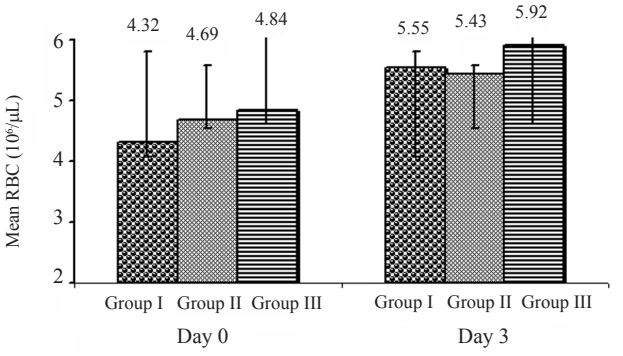

Fig. 2. The total RBC counts of rabbits treated with normal saline/control (Group I), 15 mg/kg ibuprofen (Group II) and 30 mg/kg ibuprofen (Group III)

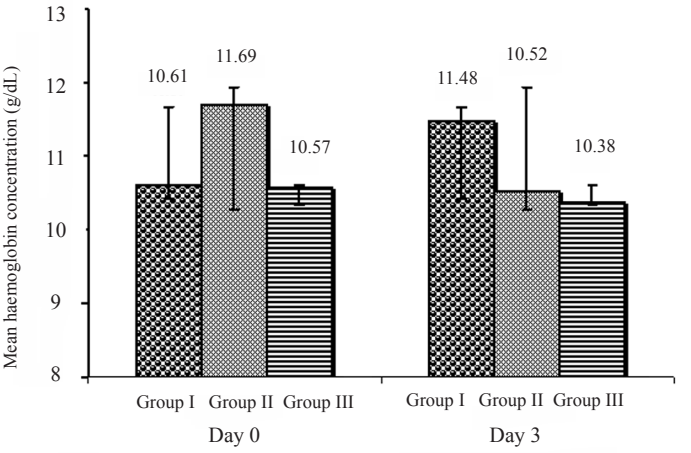

Fig. 3. The haemoglobin concentration of rabbits treated with normal saline/control (Group I), 15 mg/kg ibuprofen (Group II) and 30 mg/kg ibuprofen (Group III)

Discussion

Ibuprofen is currently used routinely in rodents and rabbits for the relief of mild to moderately painful conditions such as skin lesions, fight wounds and skin abscesses. Generally the dose for oral administration is between 7.5 and 30 mg/kg b.m. every 6-8 hours (JENKINS, 1987; LILES and FLECKNELL, 1992). Thus the study was carried out using ibuprofen 15 mg/kg b.m. and 30 mg/kg b.m. at a dosing interval of 12 hours. These two doses of ibuprofen were able to provide postoperative analgesia in the experimental rabbits. However, more rapid and deeper analgesia was obtained by the use of ibuprofen 30 mg/kg b.m. as seen by the lower pain score of the group III rabbits at 2 and 18 hours. This finding agrees with HOSKING and WELCHEW (1985) who reported a dose response effect following use of all analgesics.

Administration of the two doses of ibuprofen did not affect the PCV, RBC and Hbc of the rabbits. This result does not agree with RAMPRABHU et al. (2001) who reported a decrease in haemoglobin and haematocrit of dogs following Ibuprofen administration. The disparity in results of these two studies may be due to the differences in the dosing regimens used, feeding and environmental conditions

The measurement of serum activity of ALT and AST is relevant for the diagnoses of liver dysfunction (LEES et al., 1994). These enzymes are present in the liver cystosol (ALT) and mitochondria (AST). Serum AST is also found in significant quantities in the muscles and red cells (LEES et al., 1994). Thus a significant increase in the serum level

of ALT is principally due to hepatic cell membrane damage. Increased serum level of AST suggests severe hepatic damage, muscle disease or haemolysis (LEES et al., 1994). A significant increase in both AST and ALT suggests that the increase in AST might be hepatic in origin (LEES et al., 1994). Thus the increase in the serum levels of these two liver enzymes suggests mild hepatic damage. Ibuprofen and ketoprofen have been found to have the lowest reported calculated incidences of hepatotoxicity of all NSAIDs (KATZ and LOVE, 1992). Therefore hepatitis or other hepatic lesions are not expected to occur following the use of these two doses of ibuprofen in rabbits.

Creatinine levels of the ibuprofen 30 mg/kg b.m. treated rabbits were found to increase significantly. This suggested decreased renal function (LEES et al., 1994). NSAIDs affect renal physiology by inhibiting cyclo-oxygenase and the synthesis of vasodilatory prostaglandins. This may lead to acute intrarenal haemodynamic changes with possible deterioration of renal function in susceptible individuals (INSEL, 1996). No significant effect on renal function has been reported in controlled clinical trials with ibuprofen in humans without underlying renal disease (FOX and JICK, 1984; FUREY et al., 1992; KELLSTEIN et al., 1999). Thus, the increase in creatinine levels in these rabbits may suggest that rabbits are more sensitive to the adverse renal effects of ibuprofen. It may also be that the concurrent administration of streptomycin (10 mg/kg b.m., 5 days) enhanced the nephrototoxic effect of ibuprofen 30 mg/kg b.m.

Following ibuprofen administration no GIT side effect was observed in the rabbits. This finding agrees with MOORE et al. (1999) and DE ARMOND et al. (1995), who reported no serious GI complaint following use of prescription doses of 200 to 400 mg for 10 days in man. RAMPRABHU et al. (2001) however contradicted this finding by reporting the occurrence of melena and bloody vomiting by day 5 during the administration of 15 mg/kg b.m. and 90 mg/kg b.m. ibuprofen to dogs. Thus, it seems that the GI effect of ibuprofen varies with species and dose.

In conclusion, the two doses of ibuprofen were able to relieve laparotomy pain at the dosing regimen used. Rabbits however seem more sensitive to the adverse renal effects, thus the use of Ibuprofen 15 mg/kg b.m. should be favoured since less renal side effects were noted following its use. At the same time concurrent use of NSAIDs alongside other drugs with nephrotoxic potential should be avoided.

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SAŽETAK

Otopina ibuprofena (15 i 30 mg/kg tjelesne mase) primijenjena je oralno dvjema skupinama kunića svakih 12 sati tijekom tri dana nakon laparotomije. Određivan je učinak tih doza na jačinu boli, hematološke pokazatelje, razine različitih serumskih enzima te funkciju probavnoga sustava. Postignuti rezultati bili su uspoređeni s onima u životinja kontrolne skupine na kojima je također učinjena laparotomija. Dva sata nakon primjene 30 mg/kg tjelesne mase ibuprofena bol se značajno smanjila ($P<0,05$). Nakon 18 sati, jačina boli u kunića druge skupine (15 mg/kg) bila je značajno manja ($P<0,05$) u odnosu na kontrolnu skupinu (prva skupina). Međutim, nakon 24 sata jačina boli u kunića skupine III (30 mg/kg) i skupine II nije se značajno razlikovala ($P>0,05$). Primjena dviju doza ibuprofena nije značajno promijenila ukupan broj krvnih stanica, broj crvenih krvnih stanica i koncentraciju hemoglobina. Razine alanin-aminotransferaze (ALT) i aspartat-aminotransferaze (AST) u serumu značajno su bile povećane ($P<0,05$) u skupinama koje su dobivale ibuprofen. Značajan porast razine serumskoga kreatinina ($P<0,05$) zabilježen je u životinja kojima je dano 30 mg/kg ibuprofena. Ni u kontrolnih ni u pokusnih životinja nisu zabilježeni melena, povraćanje ni proljev. Zaključno se može reći da su dvije doze ibuprofena dovoljne da se smanji bol uzrokovana laparotomijom. Preporučuje se doza ibuprofena od 15 mg/kg tjelesne mase jer su se pri tom javljale slabije nuspojave od strane bubrega.

Ključne riječi: ibuprofen, kunići, laparotomija, analgezija, hematologija, serumski enzimi