COMPARISON OF ANTINOCICEPTIVE EFFECTS OF ANALGESICS IN A CHEMICAL MODEL OF VISCERAL PAIN IN MICE

Osman Ekinci MD,[1] Tuba Pelit MD,[1] Fethi Gül MD,[1] Dilek Subaşı MD,[1] Neşe Aydın MD,[1] Berna Terzioğlu MD,[2] Zafer Gören MD. PhD[3]

[1] Haydarpaşa Numune Research and Education Hospital, Department of Anesthesiology, Haydarpaşa, İstanbul

[2] Haydarpaşa Numune Research and Education Hospital, Department of Clinical Biochemistry, Haydarpaşa, İstanbul

[3] Marmara University, School of Medicine, Department of Pharmacology and Clinical Pharmacology, Haydarpaşa, İstanbul

ABSTRACT

Objective: *There are several experimental methods to propose the efficacy of pharmacological agents to test nociception and analgesia. Acetic acid induced writhing test is an established method for studying visceral pain. The aim of this study is to compare efficacy and potency orders of tramadol, lornoxicam and paracetamol in a mice model and supply evidence for clinicians dealing with pre-emptive analgesia.*

Material and Method: *Swiss albino mice were treated either with intraperitoneal physiological saline, tramadol (10.0 mg/kg, 5.0 mg/kg and 2.5 mg/kg), lornoxicam (0.65 mg/kg, 1.30 mg/kg and 2.60 mg/kg) or paracetamol (100 mg/kg, 50 mg/kg and 25 mg/kg) 5 min before inducing the writhing test. The mice received intraperitoneal 0.2 ml 3% acetic acid solution injections and the writhes were observed and recorded for 10 min. Percent maximum possible effects and median effective doses (ED50)were calculated and the efficacy order for the three agents were compared.*

Results: *The efficacy order for the three agents was found as: Tramadol ≥ Lornoxicam > Paracetamol. Median effective doses (ED50) for the drugs were also calculated as 1.54 mg/kg, 5.20 mg/kg and 97.32 mg/kg for lornoxicam, tramadol and paracetamol respectively. The potency order was observed as: Lornoxicam > Tramadol > Paracetamol .*

Conclusion: *These results state that tramadol and lornoxicam have similar efficacy where pre-emptive role of paracetamol should be further questioned in a clinical setting.*

Key Words: *Pain, postoperative, nociception tests, analgesics* ***Nobel Med 2011; 7(2): 40-44***

ANALJEZİKLERİN ANTİNOSİSEPTİF ETKİLERİNİN KİMYASAL AĞRI MODELİ FARELERDE KARŞILAŞTIRILMASI

ÖZET

Amaç: Nosisepsiyon ve analjeziyi test etmede kullanılan farmakolojik ajanların etkililiği için deneysel metodlar bulunmaktadır. Asetik asit kıvranma testi viseral ağrı çalışma metodudur. Bu çalışmanın amacı fare modelinde tramadol, lornoksikam ve parasetamolün potens sıralamasını ve etkililiğini karşılaştırmak ve pre-emptif önleyici analjezi ile ilgilenen klinisyenlere bilgi sağlamaktır.

Materyal ve Metod: Swiss albino fareler, kıvranma testinden 5 dak önce intraperitoneal fizyolojik tuzlu su, tramadol (10,0 mg/kg, 5,0 mg/kg ve 2,5 mg/kg), lornoksikam (0,65 mg/kg, 1,30 mg/kg ve 2,60 mg/kg) veya parasetamol (100 mg/kg, 50 mg/kg ve 25 mg/kg) ile tedavi edilmişlerdir. Farelere intraperitoneal 0,2 ml 3% asetik asit solüsyonu enjeksiyonu yapılmış ve kıvranmalar gözlemlenerek 10 dak kayıt edilmiştir. Maksimum olası etki yüzdesi ve etkin dozları (ED50) hesaplanmış ve üç ajanın etkililik sıralaması karşılaştırılmıştır.

Bulgular: Üç ajanın etkilik sıralaması şu şekilde bulunmuştur: Tramadol ≥ Lornoksikam > Parasetamol. İlaçlar için etkin dozlar (ED50) da lornoksikam, tramadol ve parasetamol için sırasıyla şu şekilde hesaplanmıştır: 1,54 mg/kg, 5,20 mg/kg ve 97,32 mg/kg. Potens sıralaması Lornoksikam > Tramadol > Parasetamol olarak gözlenmiştir.

Sonuç: Bu bulgular tramadol ve lornoksikamın benzer etkililiğe sahip olduğunu, parasetamolün ise pre-emptif rolünün klinikte araştırılması gerektiğini göstermektedir.

Anahtar Kelimeler: Ağrı, postoperatif, nosisepsiyon testi, analjezikler **Nobel Med 2011; 7(2): 40-44**

INTRODUCTION

The efficacy of pain control is an important component of post-operative care. Pre-emptive analgesia refers to maintaining intraoperative antinociception with the agents administered before surgery that also prevents the central sensitization that occurs due to injury produced by the surgical intervention.[1] The inventory of drugs used for controlling post-operative pain changes quite frequently. The most suitable post-operative pain treatment may be achieved with an agent that acts quickly and potently with minimal adverse effects and produces fewer drug interactions.[2] In fact, pre-emptive analgesia is such a complex and controversial topic that it can be viewed from many different aspects. There are several experimental methods to propose the efficacy of pharmacological agents to test nociception and analgesia. In tail-flick and hot-plate tests, the response to the stimulation is fixed and the test measures mainly the threshold of the stimulus required to elicit a response.[3] In the second type experiments, the stimulus is standardized and the strength or duration of the response is measured where formalin or acetic acid induced writhing tests may be good examples.[3] Stimuli that have been employed in studies of visceral pain can be categorized into four general groups: electrical stimuli, mechanical stimuli, chemical stimuli, and ischemia. As it has been accepted, damage to visceral tissues does not invariably lead to pain and stimuli that are not tissue damaging (or even predictive of tissue damage) can and do lead to visceral pain. So in visceral pain model, visceral stimulus must produce particular results to be termed noxious. In other models where visceral stimuli that utilize invasive surgical procedures to place stimulation equipment require use of anesthetics or analgesics the writhing test becomes a more suitable method screening the effects of analgesics.[4]

Lornoxicam, a member of the oxicam group of nonsteroidal anti-inflammatory drugs (NSAIDs) is being used successfully in prevention and treatment of post-operative pain.[5] Its inhibitory effects on cyclooxygenases in peripheral tissues decrease prostaglandin production and its effects on endogenous dynorphins, □-endorphin levels also promote its central analgesic and anti-inflammatory effects. Recent studies also suggested a pre-emptive role for lornoxicam.[6,7] Lornoxicam was reported to be more potent than other NSAIDs.[8] Several studies also showed that lornoxicam diminished post-operative opioid requirements.[5,9] Paracetamol, a drug without inhibiting peripheral cyclooxygenase-1, has a well-established safety and analgesic profile and it has few contraindications and lack significant drug interactions.[10] Intravenous paracetamol was reported to be similar to propacetamol and consistently superior to placebo for the main efficacy criterion of pain relief and intensity where intravenous acetaminophen had significantly improved local tolerability.[10,11]

The aim of this study is to supply evidence for anti-nociceptive effects of two NSAIDs and compare →

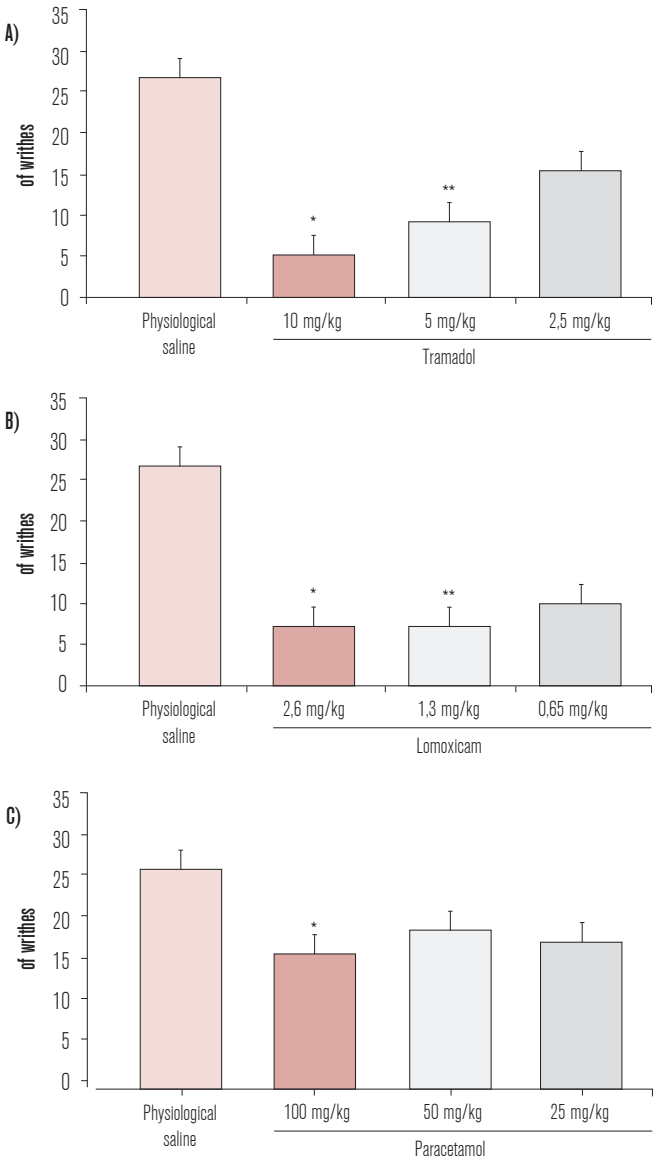

Figure 1. The effect of 3 different doses of intraperitoneal tramadol (A; 2.5, 5 and 10 mg/kg), lornoxicam (B) and paracetamol (C) on the number of writhes induced by intraperitoneal 3% acetic acid. Pre-treatments were given 15 min prior of acetic acid injections (n =7/group). Writhes were observed for 10 min. *, $p < 0.001$ and **, $p < 0.01$ (compared to physiological saline, Dunns multiple comparison test)

the results with that of tramadol, a centrally acting analgesic whose mechanism of action is predominantly based on enhanced serotonergic neurotransmission in mice by using acetic acid induced writhing test.[12]

MATERIAL AND METHOD

Animals

Swiss albino mice weighing 20-35 g of both sexes supplied from Marmara University Experimental Animal and Research Laboratory were used in the study. All procedures were approved by the "Animal Ethical Committee of Haydarpaşa Numune Education and Research Hospital". The mice were kept at 12 h light and dark cycle and fed with standard mice chow, water ad libitum.

Writhing test

The writhing test is typically carried out in unanesthetized mice using an intraperitoneal injection of fixed dose (0.2 ml/mouse) of diluted acetic acid (3% V/V) solution. Responses have been quantified as all-or-none responses where the number of writhes is counted in 10-min. A full writhe is accepted as stretching of hind limbs and dorsiflexion of hind paws frequently with the pelvis rotated sideward and contraction of abdominal muscles followed by stretching of the body and extension of the hind limbs (the classic writhing response).

Experimental protocols

The mice were treated either with intraperitoneal physiological saline, tramadol (10.0 mg/kg, 5.0 mg/kg and 2.5 mg/kg), lornoxicam (0.65 mg/kg, 1.30 mg/kg and 2.60 mg/kg) or paracetamol (100 mg/kg, 50 mg/kg and 25 mg/kg) 5 min before inducing the writhing test. The mice received intraperitoneal 0.2 ml 3% acetic acid solution injections and they were placed in plexiglass cages 20 X 20 X 20 cm.

Drugs

3% glacial acetic acid solution was prepared with physiological saline and the pH was detected to be 4. Tramadol, paracetamol and lornoxicam serial dilutions were also prepared with physiological saline.

Data processing and statistical analysis

The data are expressed as means ± SEM. Percent maximum possible effects (%MPE) were calculated according to the formula below: %MPE = [(# of writhes in each experiment - # of writhes in control group)/# of writhes in control group] X 100 Median effective doses (ED_{50} values) were calculated by using %MPE values obtained with 3 doses with the aid of linear regression. The number of writhes in different groups was compared by using *Kruskal Wallis* statistics followed by *Dunns post-hoc* test. All statistics were performed by using GraphPad Prism (USA). Statistical significance was accepted where $p<0.05$.

RESULTS

The effect of physiological saline, tramadol, lornoxicam and paracetamol on writhes induced by acetic acid. When the mice were pre-treated with intraperitoneal tramadol at 2.5, 5.0 and 10.0 mg/kg doses, Kruskal-Wallis statistics showed a significant difference between physiological saline and tramadol ($p<0.05$). Tramadol at 2.5 mg/kg dose failed to suppress the writhes, but 5 mg/kg and 10 mg/kg doses were →

found to decrease the number of writhes significantly ($p<0.01$ and $p<0.001$, respectively) as shown in Fig.1A. Comparison of effective doses of tramadol did not yield a statistically significant difference. Likewise lornoxicam pre-treatment was found to be effective compared to physiological saline ($p<0.05$) where 0.65 mg/kg dose was found ineffective in suppressing the writhes. We observed that lornoxicam suppressed the writhes significantly at 1.3 and 2.6 mg/kg doses ($p<0.01$ and $p<0.001$, respectively). No difference was detected between the effective doses (Fig.1B). Paracetamol suppressed the writhes significantly only at 100 mg/kg dose ($p<0.05$) where other doses (50 and 25 mg/kg) were found to be ineffective (Fig.1C).

The efficacy and the potency order of tramadol, lornoxicam and paracetamol %MPE's were also calculated (Fig.2) where the efficacy order for the three agents was found as follows: Tramadol ≥ Lornoksikam > Paracetamol ED_{50} for the drugs were also calculated by using the MPE's (%) of each experiment where ED_{50} values were found as 1.54 mg/kg, 5.20 mg/kg and 97.32 mg/kg for lornoxicam, tramadol and paracetamol respectively. The potency order was observed as: Lornoxicam > Tramadol > Paracetamol

DISCUSSION

In this study we wanted to compare the antinociceptive effects of three different agents independent of their mechanism of action in mice model of visceral pain. We found that efficacy of tramadol was more or equal to lornoxicam. The experiments performed in animals regarding pre-emptive analgesia may supply evidence for clinical trials to find a more suitable agent that exerts immediate and late effects on post-operative pain. We detected that tramadol exhibited a greater efficacy in writhing test in mice. Previous studies showed that paracetamol displayed a similar ED_{50} value in orofacial formalin test where it was reported to be 100.66 mg/kg.[13]

Clinical studies also showed that tramadol when administered prior to lumpectomy or before the end of the surgical procedures can produce effective postoperative analgesia.[14] Local administration of opioids was shown to have a peripheral antinociceptive effect especially in inflammatory pain. The effect of tramadol was shown in carrageenan-induced inflammatory pain in knee joints of rats.[15] The results of this research show that not only morphine but also tramadol another widely used opioid in clinical practice inhibits nociception, edema and functional impairment of the paw after its local application directly to the inflamed knee joint. It is postulated that

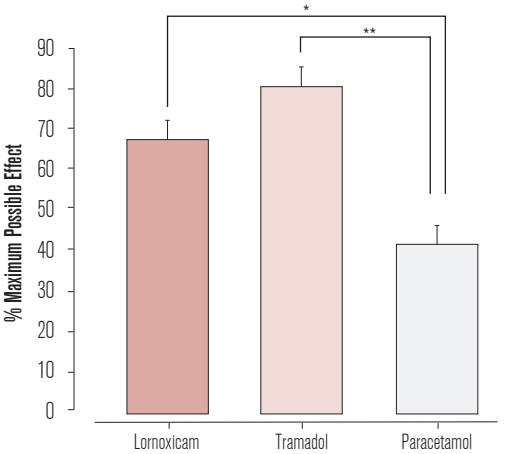

Figure 2. The comparison of % maximum possible effects of lornoxicam, tramadol and paracetamol in writhing test induced with intraperitoneal 3% acetic acid injection. (Kruskal Wallis Test, $p < 0.05$) *, $p < 0.05$, **, $p < 0.0001$

tramadol exerts its actions through two mechanisms: opioid and inhibition of central monoaminergic descending pathways.[16,17] The activation of serotonergic and noradrenergic component of descending antinociceptive system is responsible for about 60% of antinociceptive effect of tramadol involving activation of descending antinociceptive systems and suppression of reuptake of amines.[16,18] This was confirmed by the studies of Kayser et al. who demonstrated the implication of noradrenergic component in tramadol actions.[19] Rojas-Corrales et al. also showed that tramadol inhibits serotonin reuptake in the model of acute pain.[20] Most of the clinical trials suggested a pre-emptive analgesic role for tramadol.[14] Comparison of timing of administration tramadol showed that it was more effective when administered before surgery rather that peri-operative or post-operative applications.[21] In a study where the impact of tramadol and morphine on pre-emptive analgesia was examined in herniorrhaphy intervention, it was found that pre-surgical caudal morphine or tramadol reduced peri-operative sevoflurane requirements and either pre-surgical or post-surgical caudal morphine did not make any difference to post-operative analgesia.[22] Lornoxicam is being used successfully in prevention and treatment of post-operative pain.[5] Among the non-steroidal anti-inflammatory drugs lornoxicam and paracetamol were found as less efficacious when compared to tramadol. Lornoxicam is a member of the oxicam group of nonsteroidal anti-inflammatory drugs (NSAIDs). Its inhibitory effects on COX-I and COX-II in peripheral tissues decrease prostaglandin and the effects on endogenous dynorphins, β-endorphin levels also promote its central analgesic and anti-inflammatory effects. Recent studies also suggested a pre-emptive role for lornoxicam.[6,7] Lornoxicam was reported to be more potent than other NSAIDs.[8] →

Several studies also showed that lornoxicam diminished post- operative opioid requirements.[5,9] The preemptive role of lornoxicam was also demonstrated in rats where it was administered intrathecally in formalin induced foot swelling test.[23]

Paracetamol, a drug without inhibiting peripheral cyclooxygenase-1, has a well-established safety and analgesic profile and it has few contraindications and lack significant drug interactions.[10] Intravenous paracetamol was reported to be similar to proparacetamol and consistently superior to placebo for the main efficacy criterion of pain relief and intensity where intravenous acetaminophen had significantly improved local tolerability.[10,11] However placebo controlled studies may be misleading, where randomized controlled studies should be performed. In a clinical trial, it was reported that 1 g paracetamol given in major abdominal surgery did not produce pain relief and diminished post-operative analgesic requirements.[8] Some contradictory results were also reported stating that pre-operative single dose paracetamol was effective in acute post-operative analgesia and that route of administration did not change the outcomes.[24] Studies comparing pre-emptive effects of tramadol and lornoxicam yielded same results.[25,26]

In conclusion, this current study suggests that lornoxicam and tramadol has similar pre-emptive effect in acetic acid induced writhing test in mice and clinical trials should be conducted to find the more efficacious agent that can be administered before surgery.

C **CORRESPONDING AUTHOR:** Osman Ekinci MD, Haydarpaşa Numune Research and Education Hospital, Dept. of Anesthesiology, Haydarpaşa, Istanbul droekinci@mynet.com

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REFERENCES

1. Jorgen BD, Steen M. Pre-emptive analgesia. British Medical Bulletin 2004; 71: 13–27.
2. Dahl JL, Gordon D, Ward S, et al. Institutionalizing pain management: The post-operative pain management quality improvement project. J Pain 2003; 4: 361-371.
3. Pierre Beaul. Experimental pre-emptive analgesia: what value for the clinician? Canadian Journal of Anesthesia 2001; 48: 946-952.
4. Ness TJ. Models of Visceral Nociception. ILAR Journal 1999; 40: 3.
5. Kocaayan E, Ozkardeşler S, Ozzeybek D, Bayindir S, Akan M. Comparison of effects of preoperatively administered lornoxicam and tenoxicam on morphine consumption after laparoscopic cholecystectomy. Eur J Anaesthesiol 2007; 24: 714-719.
6. Karaman Y, Kebapci E, Gurkan A. The pre-emptive analgesic effect of lornoxicam in patients undergoing major abdominal surgery: a randomised controlled study. Int J Surg 2008; 6: 193-206.
7. Trampitsch E, Pipam W, Moertl M, et al. Pre-emptive randomized, double-blind study with lornoxicam in gynecological surgery. Schmerz 2003; 17: 4-10.
8. Borisov DB, Levin AV, Vyl'iurov IV, Sokolov AV, Nedashkovskii EV. Efficiency of pre-emptive intravenous paracetamol analgesia in abdominal surgery. Anesteziol Reanimatol 2007; 5: 38-40.
9. Xuerong Y, Yuguang H, Xia J, Hailan W. Ketamine and lornoxicam for preventing a fentanyl-induced increase in postoperative morphine equirement. Anesth Analg 2008; 107: 2032-2037.
10. Day RO, Graham GG, Whelton A. The position of paracetamol in the World of Analgesics. Am J Ther 2000; 7: 51–54.
11. Schiødt FV, Rochling FA, Casey DL, Lee WM. Acetaminophen toxicity in an urban county hospital. N Engl J Med 1997; 337: 1112–1117.
12. Schumacher MA, Basbaum AI, Way WL. Opioid analgesics and antagonists. In: Katzung BG (ed) Basic and clinical pharmacology, 10th ed. Singapore: Mc Graw Hill Education (Asia) 2007; 489-507
13. Luccarini P, Childeric A, Gaydier AM, et al. The orofacial formalin test in the mouse: a behavioral model for studying physiology and modulation of trigeminal nociception. J Pain 2006; 7: 908-914.
14. Shen X, Wang F, Xu S, et al. Comparison of the analgesic efficacy of pre-emptive and preventive tramadol after lumpectomy. Pharmacol Rep 2008; 60: 415-421.
15. Garlicki J, Dorazil-Dudzik M, Wordliczek J, Przew ocka B. Effect of intraarticular tramadol administration in the rat model of knee joint inflammation. Pharmacol Rep 2006; 58: 672-679.
16. Raffa RB, Friderichs E, Reimann W, et al. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J Pharmacol Exp Ther 1992; 260: 275-285.
17. Driesen B, Reimann W, Giertz H. Effects of the central analgesic tramadol on the uptake and release of noradrenaline and dopamine in vitro. Br J Pharmacol 1993; 108: 806-811.
18. Frink MC, Hennies HH, Englberger W, Haurand M, Wilffert B. Influence of tramadol on neurotransmitter systems of the rat brain. Arzneimittelforschung 1996; 46: 1029–1036.
19. Kayser V, Besson JM, Guilbaud G. Evidence for a noradrenergic component in the antinociceptive effect of the analgesic agent tramadol in an animal model of clinical pain, the arthritic rat. Eur J Pharmacol 1992; 224: 83-88.
20. Rojas-Corrales MO, Ortega-Alvaro A, Gibert-Rahola J, Roca-Vinardell A, Micó JA. Pindolol, a beta-adrenoceptor blocker/5-hydroxytryptamine (1A/1B) antagonist, enhances the analgesic effect of tramadol. Pain 2000; 88: 119–124.
21. Wordliczek J, Banach M, Garlicki J, Jakowicka-Wordliczek J, Dobrogowski J. Influence of pre- or intraoperational use of tramadol (pre-emptive or preventive analgesia) on tramadol requirement in the early postoperative period. Polish J Pharmacology 2002; 54: 693-697.
22. Ozcengiz D, Gunduz M, Ozbek H, Isik G. Comparison of caudal morphine and tramadol for postoperative pain control in children undergoing inguinal herniorrhaphy. Paediatr Anaesth 2001; 11: 459-464.
23. Yang BX, Chen X, Song L. Effect of preemptive intrathecal lornoxicam on foot swelling of formalin test in rats. Sichuan Da Xue Xue Bao Yi Xue Ban 2009; 40: 533-535.
24. Moller PL, Sindet-Pedersen S, Petersen CT, et al. Onset of acetaminophen analgesia: comparison of oral and intravenous routes after third molar surgery. Br J Anaesth 2005; 94: 642-648.
25. Petrova VV, Osipova NA, Beresnev VA, et al. Lornoxicam (xefocam) as an agent ort he prevention and treatment of postoperative pain among other nonsteroidal anti-inflammatory drugs. Anesteziol Reanimatol 2005; 5: 39-43.
26. Staunstrup H, Ovesen J, Larsen UT, et al. Efficacy and tolerability of lornoxicam versus tramadol in postoperative pain. J Clin Pharmacol 1999; 39: 834-841.