PREEMPTIVE ANALGESIC EFFECT OF DICLOFENAC: EXPERIMENTAL STUDY IN RATS

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Abstract

Background: Preemptive analgesia is an antinociceptive treatment that prevents central sensitization. Antinociceptive effects of diclofenac are well-known. The aim of this study was to investigate preemptive analgesic effects of intraperitoneally administrated diclofenac, *before* and *after* acute and inflammatory induced pain in rat model.

Methods: Forty eight male Sprague Dawley rats were included in the study. The rats are divided in five groups (n = 8 per each group); Group A, diclofenac at 10 mg/kg given *ip*, 30 min *before* the nociceptive stimulus realized with hot plate test; Group B, diclofenac at 10 mg/kg given *ip*, 5 min *after* the nociceptive stimulus, realized with hot plate test; Group C, diclofenac at 10 mg/kg given *ip*, 30 min *before* the nociceptive stimulus realized with formalin test, and; Group D, diclofenac at 10 mg/kg given *ip*, 5 min *after* the nociceptive stimulus, realized with formalin test. Saline was used as a control. Paw movements in response to induced pain with hot plate test and formalin test were measured during 60 minutes.

Results: Preemptive analgesic effect was significant in both groups when diclofenac was administrated *before* the pain stimuli (P<0.01 and P<0.001). The significant decrease in paw movements started in 15 min after pain stimuli in group A and in 25 min, in group C.

Conclusion: Intraperitoneally administered diclofenac had preemptive analgesic effects on acute thermal, and inflammatory induced pain in rats. Our results contain the preemptive analgesic effect of systematically administrated diclofenac.

Keywords: diclofenac, preemptive analgesia, hot plate test, formalin test.

Introduction

Preemptive analgesia suggests that the application of analgesic in prior to proceeding of noxious stimuli prevent the sensibility of the central nervous system, which provokes the pain. Pain associated with tissue damage results in prolonged modulation of the somatosensory system, with increased responsiveness of both peripheral and central pain pathways[1]. Experimental evidence proposes that to 'prevent' or 'preempt' the noxious input to the CNS, may be more effective than treatment. The idea of preemptive analgesia was first introduced into clinical practice by Crile

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in 1913[2] and further developed by Wall[3] and Woolf[4]. The definition of preemptive analgesia was formed by Kissin[5]. According to him, preemptive analgesia is "treatment that prevents establishment of central sensitization caused by incisional and inflammatory injuries; it starts before incision and covers both the period of surgery and the initial postoperative period. Preemptive analgesia prevents pathologic pain that is different from physiologic pain", which means: prevention or reversal of central and peripheral sensitization.

Diclofenac sodium, 2-[(2,6-dichlorophenyl) amino] benzene acetic acid, is a non-steroidal anti-inflammatory drug (NSAID), with an approximate relative COX-1/COX-2 specificity ratio of one[6]. NSAIDs inhibit the cyclo-oxygenase enzymes (COX), and decrease peripheral and central prostaglandin production. To reduce the inflammation that accompanies tissue injury, decreasing prostaglandin production attenuates the response of the peripheral and central components of the nervous system to noxious stimuli and reduce the pain occurred in response to following noxious stimuli[7]. These properties would seem to make NSAIDs ideal drugs to use in a pre-emptive approach.

Preemptive analgesic effect of diclofenac is discussed in many studies, but the results are still controversy[8-14].

In the present study the preemptive analgesic properties of systemically administered diclofenac sodium were investigated in a rat model of acute and inflammatory pain.

Materials and Methods

After Institutional Ethics Committee approval, 48 male (8 for each group) Sprague Dawley rats, weighing 250-300g (60-70 days after birth), were included in the study. The animals were housed in a cage at 20-25ºC under diurnal light condition and allowed to access food and water *ad libitum*. All experiment was done in accordance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 80-23, revised 1996).

Diclofenac sodium (Proanalysis-Merck, Germany) was dissolved in normal saline to achieve solutions of 10 mg/kg for intraperitoneally (*ip*) administration. The total injected volume was adjusted to 10 ml/kg in each rat. Diclofenac was used for *ip* injection at 1-ml syringe with a 23-G needle.

The rats are divided in five groups;

Group A, diclofenac at 10 mg/kg given *ip*, 30 min *before* the nociceptive stimulus realized with hot plate test;

Group B, diclofenac at 10 mg/kg given *ip*, 5 min *after* the nociceptive stimulus, realized with hot plate test;

Group C, diclofenac at 10 mg/kg given *ip*, 30 min *before* the nociceptive stimulus realized with formalin test, and;

Group D, diclofenac at 10 mg/kg given *ip*, 5 min *after* the nociceptive stimulus, realized with formalin test.

Saline group, (1ml/kg), normal saline given *ip*, was used as a control.

The acute thermal pain was realized with hot plate test. The hot plate test was performed at 55ºC on the paw of each rat. Animals were placed on the heated smooth surface and observations began and continued for the next 60 minutes (Fig. 1a. and 1b). To prevent the tissue injury the rats were removed from the hot plate test after 30 sec. Hot plate tests were performed 10 min before or 30 min after *ip* drug injection, and repeated every 10 min during 60 min.

Fig. 1a
Hot plate test

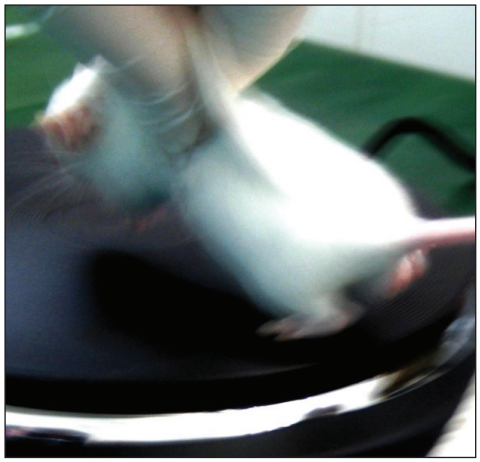
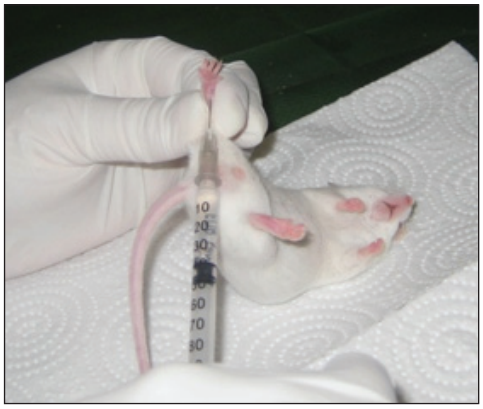


Fig. 1b
Paw movements

Fig. 1c
Formalin test



The formalin test, model of inflammatory pain, was performed ten minutes after drug administration. Fifty microlitres of 10% formalin was injected subcutaneously into the dorsal surface of the right hind paw with a 30-G needle (Fig. 1c). Immediately after injection, the rat was placed in an open surface, and their paw response was observed at ten minutes intervals for a period of one hour. The number of movements was counted for one minute. Two phases were observed: phase 1 for the first six minutes after injection; and phase 2 beginning after about ten minutes.

The paw movements were measured every 10 minutes during 60 minutes. A nociceptive score was determined by measuring the 4 behavioural categories: 0, the position and posture of the hind paw is indistinguishable from the contralateral paw; 1, the paw has little or no weight placed on it; 2, the paw is elevated and is not in contact with any surface; 3, the paw is licked, bitten or shaken.

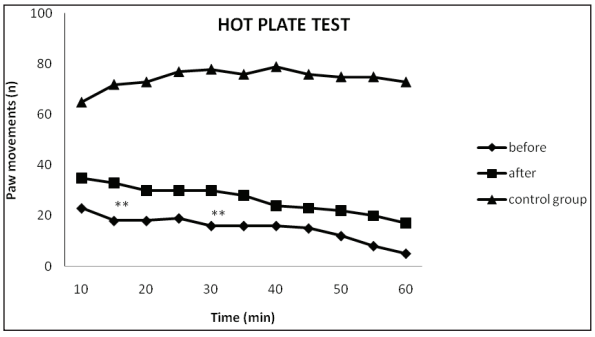
Behavioral side effects (agitation, allodinia, catatonic excitement and flaccidity) were observed in animals during the study.

Statistical analysis was performed using SPSS version 15. Data are expressed as the mean ± SD or 95% confidence interval (CI). The experimental groups were compared by the nonparametric Kruskal-Wallis test. Multiple comparisons after the Kruskal-Wallis test were performed using the two-tailed Dunn test. A *P* value less than 0.05 were considered significant.

Results

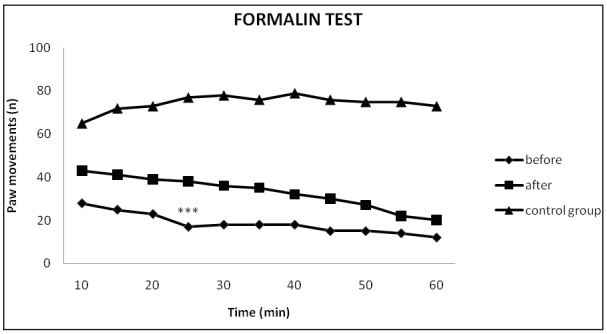
Preemptive analgesic effect, in groups *before* and *after* diclofenac sodium administration, was shown in hot plate test with significant decrease in paw movements in group A, when diclofenac sodium was administrated before the pain stimuli with hot plate test ($P<0.01$) (Fig. 2).

Fig. 2
*Paw movements after ip administration of diclofenac before and after the pain stimuli and control group in hot plate test. Data are presented as mean ± SD. * significant difference*



Significant decrease of paw movements was shown in formalin test as well, in groups *before* administration of diclofenac sodium (group C) ($P<0.001$) (Fig. 3).

Fig. 3
*Paw movements after ip administration of diclofenac before and after the pain stimuli and control group, with formalin test. Data are presented as mean ± SD. * significant difference*

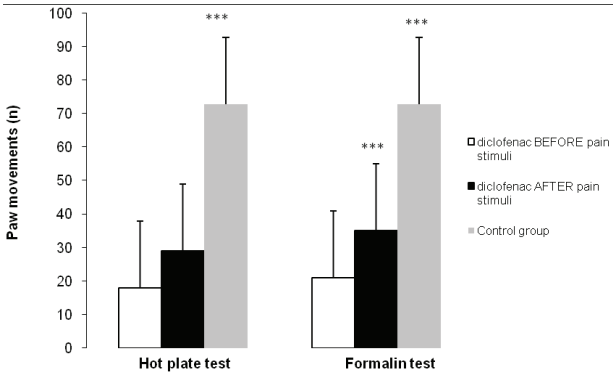


Antinociceptive effects in the hot plate test, group *before* (group A) started in 15 min after diclofenac administration and continue to decrease during followed 60 minutes. In the formalin test group *before* (group C) significantly increased paw movements, antinociceptive effects continue to decrease in 25 min and up to 60 min.

Diclofenac decrease the number of paw movement in both phases of formalin test, in group C ($P<0.05$).

Antinociceptive effects of diclofenac administrated *before* pain stimuli were higher in group C, than the corresponding group A ($P<0.01$ *vs.* $P<0.001$, respectively) (Fig. 4).

Fig. 4
*Paw movements after ip administration of diclofenac before and after the pain stimuli and control group, in hot plate test and formalin test. Data are presented as mean ± SD. * significant difference*



Both *before* and *after ip* administration of diclofenac decrease the number of paw movements after pain stimuli performed by hot plate or formalin, in comparison with control saline group ($P<0.001$) (Fig. 2, 3, 4).

No behavioural side effects were observed in any animal.

Discussion

In this recent study, we demonstrated that intraperitoneally administered diclofenac had preemptive analgesic effects in the hot plate test and formalin test in rats.

Preemptive analgesia can reduce both the acute and inflammatory pain and in this way can reduce peripheral and central sensitization.

In our study, we used *before* versus *after* design; we applied diclofenac before and after pain stimuli.

It is evident that pain processing may be reduced by preadministration of various agents (e.g. opioids, local anesthetics, NSAID etc.) leading to the concept of preemptive analgesia. Due to their mode of action, competing with arachidonic acid for binding to cyclo-oxygenase and decreasing the formation of prostaglandins, treatment with NSAIDs should be started as early as possible. The treatment should be initiated before the input of nociceptive stimuli; however, the clinical value of this technique remains still uncertain[15].

Instrumentation of the uterus and Fallopian tubes during laparoscopy or surgery leads to prostaglandin release and, the prostaglandins released play a role in pain following laparoscopy[16]. Inhibition of prostaglandin production by the NSAIDs both peripherally and centrally should, therefore, decrease postoperative discomfort and reduce opioid requirement[17].

Woolf and colleagues showed no difference with preoperative diclofenac from postoperative diclofenac in patients undergoing laparoscopic tubal ligation[18].

However, Buggy et al. and Gillberg et al. demonstrate that preoperative administration of ketorolac, piroxicam and diclofenac did reduce postoperative pain in patients undergoing laparoscopy[19,20]. Our findings support these results as well.

There are two systematic reviews published in recent years; one of them supports the clinical value of preemptive analgesia, whereas the other one is the opposite. Firstly, Moiniche et al[21] published a systematic review of 80 studies, with 3761 patients, based on *before* versus *after* design. They show that the trials of single-dose epidural analgesia resulted with an improvement in pain control in 7 of 11 studies, but that validity and clinical relevance of the effect of epidural analgesia were uncertain. They concluded that the preemptive use of analgesics is not resulted in better postoperative pain relief. However, three years later, Ong et al. in their review analyzed 66 studies with data from 3261 patients and concluded that preemptive epidural analgesia, preemptive local wound infiltration and NSAID administration improve postoperative

pain scores[22]. Comparing the two reviews, Ong et al. suggested that 10 additional new trials from 2001–2003, not included in the review by Moiniche et al.

Our study shows that *ip* administered diclofenac has preemptive and antinociceptive effects in acute thermal and inflammatory induced pain.

The hot plate test evaluates supraspinal antinociceptive effects, and it reflects activity in thermally sensitive afferent fibers and activity of Aδ and C fibers[25].

Responses in the formalin test are mediated by both the spinal and supraspinal sites. The phase 1 response of the formalin test is caused by the direct stimulation of nociceptors by formalin or tissue damage, and is thought to be an acute pain reaction. This reflects activity that is prominent in Aβ, Aδ and high-threshold C nociceptor afferent fibers. The phase 2 response is caused by inflammation after formalin injection and central sensitization related to C activity. It reflects activity in mechanically insensitive afferent fibers and activity of Aδ and C fibers[26].

Sensory fibers respond to physical and chemical stimuli producing mediators with origin from tissue injury and inflammation. These inflammatory mediators activate or sensitize afferent fibers, and the neural impulses originated from nociceptors are transmitted via peripheral nerves to the spinal cord and with cranial nerves to cranial nerve ganglia. Prostaglandins are among the most important mediators of inflammatory pain. During inflammation prostaglandin formation is induced by COX enzymes. NSAIDs block COX enzymes production and produce analgesia[27].

Studies have highlight that NSAIDs do not increase the pain threshold in animal model such as tail-flick and hotplate tests, but they normalize the pain behavior, which is observed after tissue injury and inflammation mechanism[28,29]. However, diclofenac cause dose dependant analgesia. The ED50 values for diclofenac are 7.20 mg/kg (3.95 ± 13.30)[30]. We assume that the inadequate administered doses of drug may decrease the concentration in peripheral nociceptive terminals and antinociceptive response may fail.

The intraperitoneally administrated dose of diclofenac sodium, in our study, was 10 mg/kg, the optimal dose to cause antinociceptive response in rats.

The analgesic action of NSAIDs is generally considered to be related to an inhibition of the enzyme cyclo-oxygenase and to involve a peripheral site of action. Recent studies have demonstrated the central antinociceptive mechanism of action of the NSAIDs[31-36].

Bjorkman studied the site and nature of the antinociceptive effect of diclofenac and paracetamol in the central nervous system. He observed the antinociceptive effect of diclofenac engage with central nervous component in different areas of central nervous system[30].

Herrero and colleagues[31] studied the central antinociceptive effect of NSAID ketoprofen in two experiment models in rats and conclude that ketoprofen has central while peripheral analgesic activity.

The present results suggest that intraperitoneally administered diclofenac has few effects at the level of the spinal cord and antinociceptive effects in the periphery and the brain.

However the available preoperative trials of preemptive NSAID use have modest or unclear results and it may be due to controversy associated with the definition of preemptive analgesia. Even though, NSAIDs may have an ability to induce a preemptive analgesic effect. Our study suggests how the preoperative use of diclofenac was more effective. It is expected that NSAIDs will play an increasing role in preemptive analgesia and pain relief in general.

References

1. Woolf CJ, Chong MS: Preemptive analgesia-treating postoperative pain by preventing the establishment of central sensitization. *Anesth Analg;* 1993, 77:362-379.
2. Crile GW: The kinetic theory of shock and its prevention through anoci-association. *Lancet;* 1913, 185:7-16.
3. Wall PD: The prevention of postoperative pain. *Pain;* 1988, 33:289-290.
4. Woolf CJ: Central mechanisms of acute pain. In Bond MR, Charlton JE, Woolf CJ (eds) *Proc. 6th World Congr on Pain;* 1991, Amsterdam: Elsevier, 25-34.
5. Kissin I: Preemptive analgesia. *Anesthesiology;* 2000, 93:1138-43.
6. Van Der Marel CD, Anderson BJ, Romsing J, Jacqz-Aigrain E, Tibboel D: Diclofenac and metabolite pharmacokinetics in children. *Paediatr Anaesth;* 2004, 14:443-51.
7. Kokki H: Nonsteroidal anti-inflammatory drugs for postoperative pain: a focus on children. *Paediatr Drugs;* 2003, 5:103-123.
8. Riad W, Moussa A: Preoperative analgesia with rectal diclofenac and/or paracetamol in children undergoing inguinal hernia repair. *Anaesthesia;* 2007, 62:1241-1245.
9. Tuzuner AM, Ucok C, Kucukyavuz Z, Alkis N, Alanoglu Z: Preoperative diclofenac sodium and tramadol for pain relief after bimaxillary osteotomy. *J Oral Maxillofac Surg;* 2007, 65:2453-8.
10. Alexander R, El-Moalem HE, Gan TJ: Comparison of the morphine-sparing effects of diclofenac sodium and ketorolac tromethamine after major orthopedic surgery. *J Clin Anesth;* 2002, 14:187-92.
11. Yukawa Y, Kato F, Ito K, Terashima T, Horie Y: A prospective randomized study of preemptive analgesia for postoperative pain in the patients undergoing posterior lumbar interbody fusion continuous subcutaneous morphine, continuous epidural morphine, and diclofenac sodium. *Spine*; 2005, 30:2357-61.
12. Jakobsson J, Rane K, Davidson S: Intramuscular NSAIDS reduce post-operative pain after minor outpatient anaesthesia. *Eur J Anaesthesiol;* 1996, 13:67-71.
13. Joshi A, Parara E, Macfarlane TV: A double-blind randomised controlled clinical trial of the effect of preoperative ibuprofen, diclofenac, paracetamol with codeine and placebo tablets for relief of postoperative pain after removal of impacted third molars. *Br J Oral Maxillofac Surg;* 2004, 42:299-306.
14. Oztekin S, Hepaguslar H, Kar AA, Ozzeybek D, Artikaslan O, Elar Z: Preemptive diclofenac reduces morphine use after remifentanil-based anaesthesia for tonsillectomy. *Paediatr Anaesth;* 2002, 12:694-699.
15. Mccrory CR, Lindahl SG: Cyclooxygenase inhibition for postoperative analgesia. *Anesth Analg;* 2002, 95:169-76.
16. Wang ZH, Wu R, Ge X: Relationships between pelvic pain and prostaglandin levels in plasma and peritoneal fluid collected from women after sterilization. *Contraception;* 1992, 45:67-71.
17. Mccormack K: Non-steroidal anti-inflammatory drugs and spinal nociceptive processing. *Pain:* 1994, 59:9-43.
18. Woolf CJ, Chong MS: Preemptive analgesia – treating postoperative pain by preventing the establishment of central sensitization. *Anesth Analg;* 1993, 77:362-79.
18. Buggy DJ, Walll C, Carton EG: Preoperative or postoperative diclofenac for laparoscopic tubal ligation. *Br J Anaesth;* 1994, 73:767-70.
19. Gillberg LE, Harsten AS, Stahl LB: Preoperative diclofenac sodium reduces post-laparoscopy pain. *Can J Anaesth;* 1993, 40:406-8.
20. Moiniche S, Kehlet H, Dahl JB: A Qualitative and quantitative systematic review of preemptive analgesia for postoperative pain relief. The role of timing of analgesia. *Anesthesiology;* 2002, 96:725-741.
21. Ong CK, Lirk P, Seymour RA, Jenkins BJ: The efficacy of preemptive analgesia for acute postoperative pain management: a meta-analysis. *Anesth Analg;* 2005, 100:757-73.
22. Espejo EF, Mir D: Structure of the rat's behaviour in the hot plate test. *Behav Brain Res*; 1993, 30, 56:171-6.
23. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K: The formalin test: an evaluation of the method. *Pain;* 1992, 51:5-17.
24. Dray A: Inflammatory mediators of pain. *Br J Anaesth;* 1995, 75:125-131.
25. Bjorkam R, Hedner J, Hedner T, Henning M: Central, naloxone-reversible antinociception by diclofenac in the rat. *Naunyn Schmiedebergs Arch Pharmacol*; 1990, 342:171-6.
26. Miranda HF, Lopez J, Sierralta F, Correa A, Pinard G: NSAID antinociception measured in chemical and a thermal assay in mice. *Pain Res Manage;* 2001, 6:190-196.
27. Miranda HF, Sierralta F, Pinard G: Neostigmine interactions with non steroidal anti-inflammatory drugs. *Br J Pharmacol*; 2002, 135:1591-7.
28. Bjorkman R: Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Experimental studies in the rat. *Acta Anaesthesiol Scand;* Suppl. 1995, 103:1-44.
29. Herrero JF, Parrado A, Cervero F: Central and peripheral actions of the NSAID ketoprofen on spinal cord nociceptive reflexes. *Neuropharmacology*; 1997, 36:1425-31.
30. Yaksh TL, Dirig DM, Conway CM, Svensson C, Luo ZD, Isakson PC: The acute antihyperalgesic action of nonsteroidal, anti-Inflammatory drugs and release of spinal prostaglandin E2 is mediated by the inhibition of constitutive spinal cyclooxygenase-2 (COX-2) but not COX-1. *J. Neurosci;* 2001, 21:5847-5853.
31. Mccormack K: The spinal actions of nonsteroidal anti-inflammatory drugs and the dissociation between their anti-inflammatory and analgesic effects. *Drugs;* 1994, 47 Suppl 5:28-45.
32. Jurna I: Central analgesic effects of non-steroidal anti-rheumatic agents. *Z Rheumatol;* 1991, 50 Suppl 1:7-13.
33. Bjorkman RL, Hedner T, Hallman KM, Henning M, Hedner J: Localization of the central antinociceptive effects of diclofenac in the rat. *Brain Res;* 1992, 590:66-73.