ORIGINAL ARTICLE

The analgesic effect of diclofenac sodium administered via the epidural route in an experimental visceral pain model

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Abstract

Purpose: The aim of this study was to investigate the characteristics of the analgesic effect of diclofenac sodium injected epidurally in single or repeated doses and whether tolerance develops in long-term use.

Materials and Methods: A total of 30 rats were included in the study. The rats were anesthetized using intraperitoneal ketamine hydrochloride and an epidural catheter (EC) was inserted at the level of 13th dorsal thoraco-lumbar vertebrae (T13). Eleven rats were excluded from the study. The remaining 19 rats were randomly divided into three groups; Group Control (Group C) (n = 6) received 20 μL normal saline solution (NS) via EC for 10 days; Group Single Dose (Group SD) (n = 6) received 20 μL NS for 9 days and 6 μg diclofenac via EC on 10th day; Group Ten Doses (Group TDs) (n = 7) received 6 μg diclofenac via EC in 20 μL NS for 10 days. On the 10th day, 30 min after epidural diclofenac sodium, 300 mg/kg of 3% acetic acid was injected via intraperitoneal route, and the rats were observed for 30 min and number of writhing reflex (WR) was recorded.

Results: The values of total number of Writhing Reflex (WRT) and Writhing reflex per minute(WR/min) were found to be significantly higher in Group C compared with Groups SD and TD (P = 0.009).

Conclusion: Single and repeated doses of diclofenac sodium via epidural route have an analgesic effect in a visceral pain model in rats without developing tolerance.

Key words: Diclofenac sodium, epidural administration, visceral pain

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) have long been known to exert potent anti-inflammatory, analgesic, and antipyretic actions in peripheral sites. However, an increasing body of experimental evidence indicates that such drugs also have a powerful central effect on pain, independent of their anti-inflammatory effects.[1-5] NSAIDs are believed to reduce enhanced nociceptive activity in the periphery by inhibiting the enzyme cyclooxygenase (COX) and thus lead to inhibition of prostaglandin (PG) generation which sensitizes afferent nociceptors to the effects of substances such as

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substance P and bradykinins.[1] In addition to the inhibition of PG synthesis in the periphery, a central action of NSAIDs has been suggested by experimental studies in which they have been demonstrated to act more potently by intrathecal administration than by systemic administration.[1-5] Lauretti *et al*. reported two end-stage cancer patients who used a single dose of epidural diclofenac sodium and had pain-free periods.[5] We could not obtain any information about diclofenac sodium usage via epidural route neither in rats nor in humans except for this case report of two cancer patients who had self-administered epidural diclofenac sodium without the doctor's permission. The safety of usage of a drug has to be confirmed with animal studies before use in patients. Thus, we investigated the analgesic effect of diclofenac sodium administered via the epidural route in a single dose or repeated doses in a visceral pain model in rats and also to see whether tolerance develops with repeated usage.

Materials and Methods

Approval for the study was granted by the Animal Testing Ethics Committee (Ankara Numune Education and Research Hospital/05.04.2001/number: 2). (The study was conducted in the Animal Testing Laboratory of the Pharmacy Faculty at Gazi University, Ankara, Turkey). A total of 30 male Sprague–Dawley albino rats with normal motor activities, weighing between 300 and 400 g bred in the Animal Testing Laboratory, were included in the study. After each rat was put into an individual cage with free access to food and water, feeding, temperature, environment, diurnal, and nocturnal conditions were standardized in optimum conditions. The rats were allowed to adapt to their environment for at least 2 days after delivery before any surgical procedure. After 2 days, epidural catheters (ECs) were applied.

Exclusion criteria were determined as motor function deficit development in the fore and hind legs during catheter insertion or within subsequent 5 days, or development of infection during follow-up.

Preparation of drugs

The drugs were diluted with normal saline solution (NS) so as to include 6 μg diclofenac sodium (dikloron 75 mg/3 ml ampoule, deva, Turkey) in 20 μL. EC injections were made using a 10 μL Hamilton injector (Hamilton Bonaduz AG, Bonaduz, Switzerland). First, 20 μL epidural drug was given, then 20 μL to prevent drug accumulation in dead space (by taking dead space as 18 μL).

Preparation of subjects

The rats were anesthetized with 66.5 mg/kg intraperitoneal ketamine hydrochloride (Ketalar 500 mg/10 ml flacon, Pfizer, Turkey). The rats were placed on the table in the prone position (B. Braun Appartebau Melsungen, Melsungen, Germany). EC was made with the Nishiyama and Hanaoka method[6] [Figures 1-3]. After determination of the most prominent spinal process in the dorsal thoracolumbar region (T13), a 1–2 cm midline incision was made. Following dissection of the muscles, the intervertebral space was found, and an EC was advanced 2 cm caudally. (B. Braun, Perifix Paed, 24-gauge, OD 00.60 mm, ID = 0.35 mm) [Figures 4-7]. The tip of the catheter was considered to be at L3–4 level according to previous studies by Nishiyama. Leakage control was made by injecting 20 μL SF via a Hamilton injector [Figure 8]. The remaining part of the catheter was advanced subcutaneously in the dorsum with a pediatric epidural needle (Braun Perifix Paed Needle 20-gauge). Muscles were sutured with 3-0 vicryl (Çetin Kimya Sağlık Araç ve Gereçleri ve Tic. Ltd., Şti, Adana, Turkey), the skin incision was sutured with 3-0 silk [Figures 9 and 10] (Orhan Boz Tıbbı Malzeme ve Sanayii A.Ş.Sincan-Ankara, Turkey). After recovery from anesthesia, the rats were observed for motor function, general behavior, placing-stepping reflex, and writhing reflex (WR) for 5 days. Infection prophylaxis of 10 mg/kg/24 h cefazolin sodium (Cefamezin, Eczacıbası Drug Industry and Trade Company, Istanbul, Turkey was administered intraperitoneally to all rats for 5 days.

Formation of groups and drug administrations

During the 5-day period after surgery, 11 rats developed motor deficit so were excluded from the study. The remaining 19 rats were randomly divided into three groups:

Group Control (Group C) (n = 6): Received 20 μL NS via EC for 10 days; Group Single Dose (Group SD) (n = 6): Received 20 μL NS via EC for 9 days, 20 μL NS + 6 μg diclofenac sodium via EC on 10th day.

Group Ten Doses (Group TDs) (n = 7): Received 20 μL NS + 6 μg diclofenac sodium via EC for 10 days.

The catheters were washed out with 20 μL NS following drug administrations daily. The rats were followed up in terms of motor deficit, general behaviors, placing-stepping reflex, and WR following the procedure every day. On the 10th day, 30 min after epidural diclofenac sodium administration, 300 mg/kg 3% acetic acid was injected intraperitoneally in accordance with the modified Koster method.[7] Koster *et al*. found repeated stretching movements after intraperitoneal acetic acid administration. After acetic acid administration, the rats were observed for 30 min and the number of WRs was recorded. Body weights were re-evaluated 48 h after the last drug dose.

Writhing reflex test

WR is a chemical nociceptive stimulation test, which is simple and sensitive. The basic movement is repeated contraction of abdominal muscles and subsequent

extension of the hind feet. Abdominal contractions begin a few minutes after the injection, reaching a maximum at 5–10 min. This time has been limited to 30 min in many studies.[8] Phenylquinone, ethacrynic acid, or acetic acid are widely used chemical stimulants for this purpose.[9] Lim and Guzman[10] described four components of the test: (1) Elongation of the body with concave arching of the back; (2) internal rotation of one foot with turning of one feet; (3) sucking in the belly; (4) side rolling or turning around and circling the cage. The test is primarily used to detect the potency of substances that have analgesic potential.[10,11] In this study, the writhing test was used to test the analgesic effect of diclofenac sodium administered epidurally, and it was evaluated as total number and number per minute.

Statistical analysis

Statistical analyses were conducted with Statistical Package for Social Science 11.5 (SPSS Inc, USA) package program for Windows. Normality distribution of constant variables was evaluated with the Shapiro–Wilk test. The results were expressed as a mean and standard deviation. Kruskal–Wallis and Mann–Whitney U-tests were used for intergroup comparisons.

Results

Thirty male Sprague–Dawley albino rats with normal motor activities, weighing between 300 and 400 g were observed in terms of general behaviors for 5 days following catheterization. Eleven rats were excluded from the study as motor deficit developed and the remaining 19 rats were included in the study. No motor deficit, agitation, or dysfunction were detected in the remaining rats during the study period.

Body weight values before and after the study are given in Table 1. No statistically significant difference was found with Kruskal–Wallis test in the intergroup and in-group comparisons of mean body weight values ($P = 0.924$ for body weight before the study and $P = 0.903$ for body weight 48 h after the last drug dose). Total WR values were found to be significantly higher in Group C compared with Groups SD and TD ($P = 0.009$). Although the values in

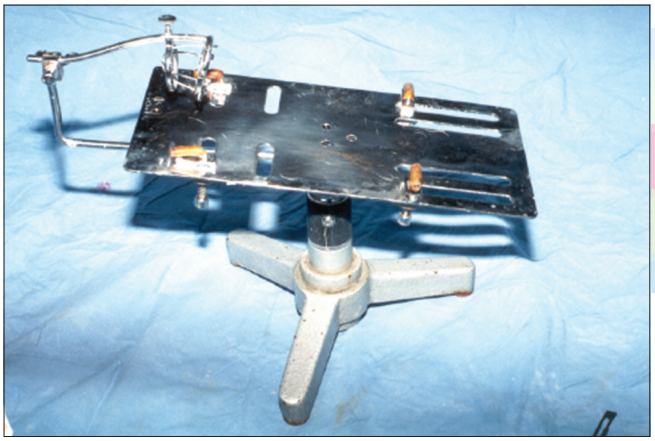

Figure 1: B. Braun Apparatebau

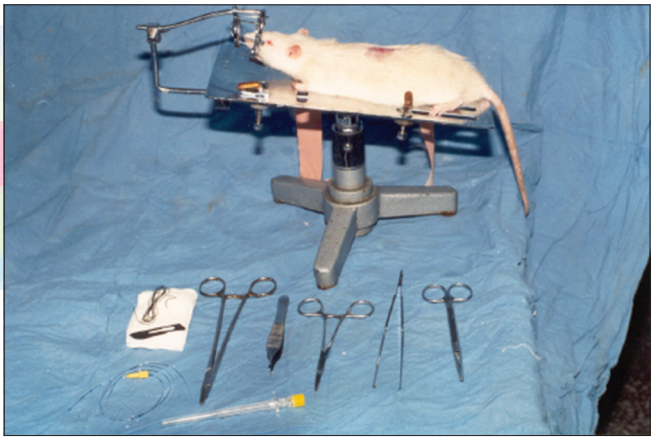

Figure 2: Position of the rat before surgical intervention

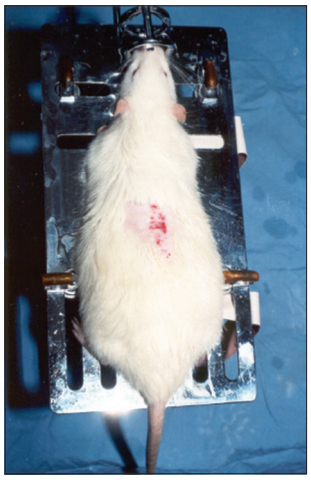

Figure 3: Surgical preparation phase

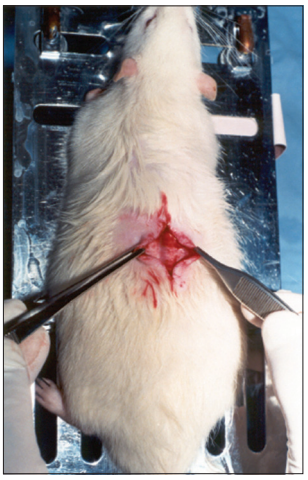

Figure 4: Midline incision

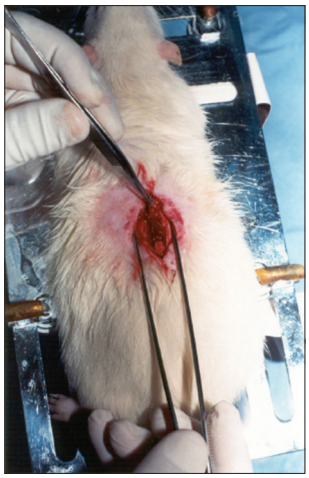

Figure 5: Detection of intervertebral space

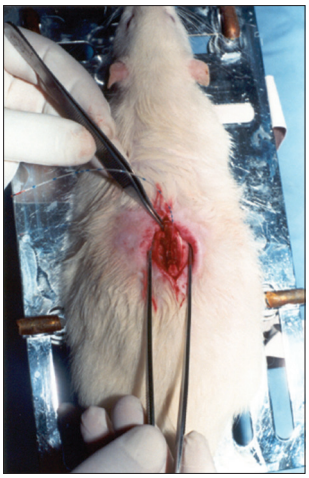

Figure 6: Catheterization

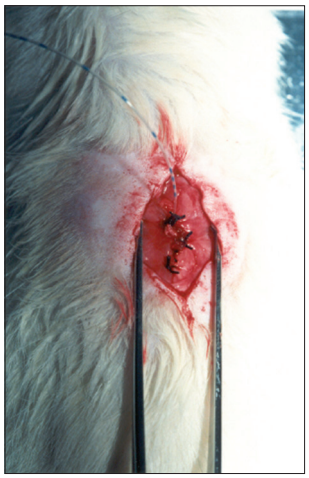

Figure 7: Closure of muscles and the fascia

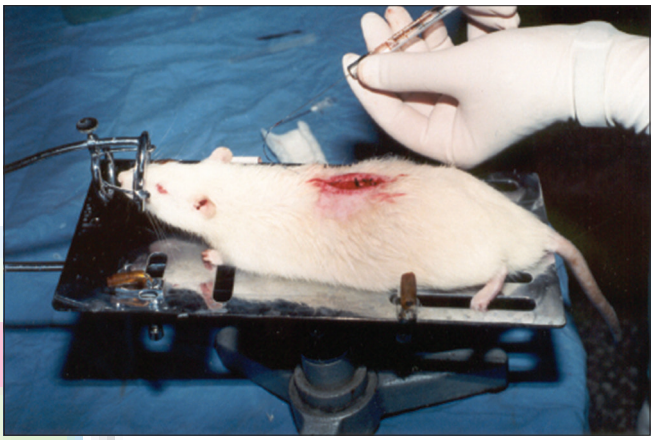

Figure 8: Leakage control with Hamilton's syringe

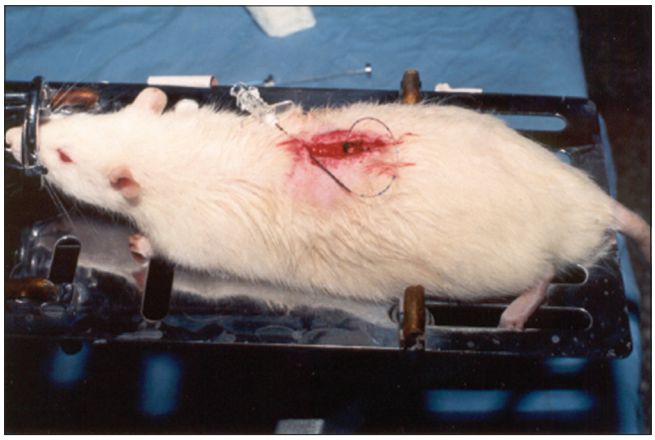

Figure 9: Subcutaneous tunnel formation for the catheter

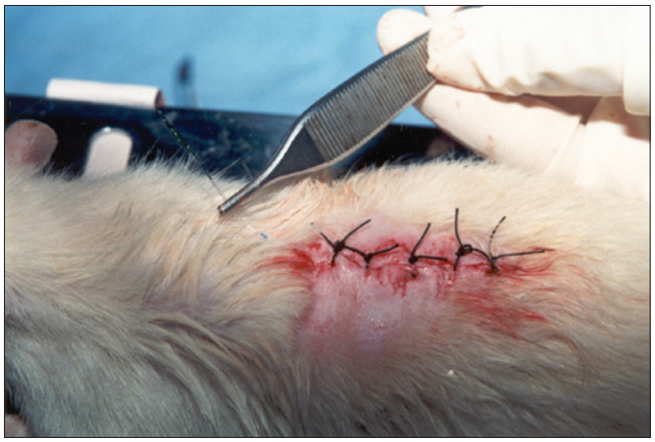

Figure 10: Occlusion of the catheter tip with a steel guidewire

Group TD were lower [Table 2], no statistically significant difference was detected between Group SD and Group TD in terms of total WR values ($P = 0.312$). WR per minute values were found to be statistically significantly higher in Group C compared with Groups SD and TD with Kruskal–Wallis test ($P = 0.009$). The WR per minute values were lower in Group TD [Table 2], but there was no significant difference between Group SD and Group TD in terms of WR per minute with Mann–Whitney U-test ($P = 0.312$).

Table 1: Body weight; in-group and inter-group comparisons

Body weight (g)	Group C (*n*=6)	Group SD (*n*=6)	Group TD (*n*=7)	*P*
Body weight (before)	362.67±17.29	361.67±17.04	362.57±17.89	0.924
Body weight (after)	363.00±16.85	362.50±17.38	363.00±16.85	0.903

Data are expressed as mean±SD. (*P*>0.05)

Table 2: Comparison of values of writhing reflex total and writhing reflex per minute between groups

	Group C (*n*=6)	Group SD (*n*=6)	Group TD (*n*=7)	*P* (paired comparison 1-2 and 1-3)
WRT[1]	14.50±3.27	9.67±3.56	7.57±1.81	0.009*
WR/min[2]	0.48±0.11	0.32±0.12	0.25±0.06	0.009*

(Data are expressed as mean±SD, [1]WRT=Writhing reflexes total; [2]WR/min=Writhing reflexes per minute

Discussion

In this study, it was observed that epidurally administered diclofenac sodium, whether a single or repeated doses of diclofenac sodium significantly decreased the WR number in a visceral pain model in both groups when compared to the control group. In the repeated dose group, the WR numbers were lower than the ones in the single dose group, but this was not statistically significant. This result also showed that tolerance to diclofenac sodium did not develop with repeated use of the drug.

Visceral pain is still an important problem and studies are ongoing for its treatment. This study was conducted on a visceral pain model for this reason. There are ways of eliciting visceral pain. Koster *et al*., Siegmund *et al*., and Jaques studied WR test to search analgesic effects of some drugs.[7-9] Acetic acid model has limitations. This reflex test can also be abolished with nonanalgesic drugs making its sensitivity lower. Björkman *et al*. studied visceral pain model both by colorectal distension and monitored heart rate and blood pressures of rats or by ethacrynic acid-induced WR test.[12-14] We preferred acetic acid induced WR test because it causes visceral pain by chemical irritation of nerve endings and peritonitis is easy to do, and it can be performed in normal standardized laboratories with no need of higher technology. Intrathecally or epidurally administered opioids produce selective spinal analgesia. However, the use of opioids by these routes involves problems such as respiratory depression and tolerance.[15] Advances in physiology and pain relief have revealed alternative pathways and neurotransmitters.[16] The development of spinal drugs that exert antinociceptive effects and minimize adverse effects has enabled their use as alternatives to opioids.[15] Therefore, many studies have been performed on the intrathecal or epidural use of NSAID.[1,12-14,17]

In a previous study, diclofenac sodium administered via subcutaneous, intracerebroventricular, and intrathecal routes was shown to lead to dose-dependent inhibition in a visceral pain model.[18] Björkman also studied the localizations of central antinociceptive effects of diclofenac sodium. The ventromedial thalamus and periaqueductal gray matter and nucleus raphe magnus were found to be the central sites of action.[12-14] In addition, c-fos protein activated in the spinal cord, gives information about neuron localization, has been shown to increase as a response to painful stimuli and diclofenac has been shown to reduce this.[19] In these studies, diclofenac sodium was considered to inhibit COX leading to inhibition of central PG and also a reduction of its biosynthesis at the spinal level.[12,20-22] Consistent with these studies, in the current study, visceral pain was also found to be reduced in the epidural diclofenac administered groups.

For NSAID administered via the epidural route to be effective in a hyperalgesia model, it has been hypothesized that they should be administered at spinal cord level.[1,22] Thus, it was hypothesized that epidural NSAID reached the spinal cord directly by being absorbed from dural membranes, passing the blood brain barrier and showing supraspinal effects by merging into the spinal cord flow through epidural veins.[1,22] In the current study, it was detected that pain diminished after epidural administration of diclofenac sodium, and therefore, we agree with the aforementioned hypothesis.

Björkman *et al*.[12-14] indicated that the analgesic effect appeared after a central diclofenac injection and naloxone administered 30 min after the diclofenac injection antagonized the analgesic effect. This finding engendered the opinion that the analgesic effect of diclofenac occurs through μ receptors directly or indirectly. In the current study, naloxone could not be administered to support this information, which may be a limitation of this study.

There are a limited number of studies about epidural administration of diclofenac sodium, the effectiveness of which has been proven with various administration routes.[1,12-14,16,18-22] There is a need for further studies about epidural administration of NSAID. In addition, the effect of epidural NSAID on substance P and pain models with NMDA and substance P in central projections of neurons appear as a topic that should be investigated, as Papworth *et al*.[23] indicated that intravenous diclofenac sodium reduced substance P.

Conclusion

We consider that single or repeated doses of diclofenac sodium administered via the epidural route is effective on a visceral pain model.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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