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Evaluation of the Antinociceptive Effect of Ketorolac in Rats

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Abstract

Currently, there is a demand for safe and non-addictive analgesics that are effective and have minimal adverse effects. The analgesic effects of ketorolac were examined in male rats to assess its potential applications in relevant domains. The median lethal dose (LD_{50}) of ketorolac, which resulted in the mortality of 50% of the experimental animals, was (693 mg/kg) by intramuscular administration. The rats showed signs of acute poisoning, represented by lethargy, elevated respiration, and Straub's tail, resulting in nervous convulsions and death within 10–15 minutes of dosing. On the other hand, the median analgesic dose (ED_{50}) that produced analgesia in 50% of the animals was (14.39 mg/kg) after I.M injection. Ketorolac exerts an analgesic effect in the acetic acid-induced writhing method. Rats administered ketorolac at multiple doses (20, 40, and 80 mg/kg,i.m.) exhibited a substantial decrease in the frequency of writhing episodes in a dose-dependent manner to 81.6 ± 1.94, 59.8 ± 1.82, and 39.4 ± 1.77, respectively, compared to the control group that received normal saline (103±4.63). This research indicate the possibility of using this treatment safely as an analgesic under the supervision of the veterinarian.

Key words: Ketorolac, Analgesic, Acetic acid, Rats

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Introduction

Ketorolac is a prevalent agent within the 1st cohort of non-steroidal anti-inflammatory agents (NSAIDs), providing therapeutic indications as analgesia, antipyretic, and anti-inflammatory properties (1,2,3). Ketorolac exerts its action by the reversible non-selective suppression of the cyclooxygenase (COX) enzymes, i.e., COX1 and COX2 isoforms, therefore obstructing the conversion of arachidonic acid to prostaglandins, which alleviates pain, fever, and inflammation (4). Ketorolac serves as a peripheral analgesic for the treatment of moderate to severe pain, exhibiting side effects (5) . It is recognised as an effective, licensed parenteral medication that is both cost-efficient and effective. In contrast to the central opioid analgesics such as tramadol and morphine, which induce severe adverse effects like respiratory depression and have potential for abuse (5), the main adverse effects of NSAIDs are on the gastrointestinal mucosa, including increased mucosal permeability, resulting in inflammation, ulceration, bleeding, and gut perforation (6,7). Ketorolac, is indicated for the treatment of acute moderate-to-severe pain (8,9). It is available in many dosage forms, including oral, nasal spray, intravenous, intramuscular, and ophthalmic solution (10,11,12,13). Ketorolac, extensively used in postoperative pain management (14,15), especially when co-administered with opioids, results in a significant decrease in opiate consumption. This combination approach not only diminishes the need for opioids but also decreases the occurrence of adverse effects, such as emesis and decreased gastrointestinal motility (15,16). In human medicine, the administration of ketorolac should not exceed 5 days due to the risk of cardiac thrombotic events, renal failure (17), peptic ulcers (18), and internal bleeding risk (7). This research seeks to ascertain the median lethal dosage (LD_{50}) and median effective dose (ED_{50}), as well as to calculate the therapeutic index, depending on the analgesic effectiveness of ketorolac in rats.

Materials and Methods

Laboratory animals

This research used mature male Albino rats weighing between 200 and 250 grams,obtained from the animal house of the College of Veterinary Medicine, Duhok University. Animals were kept under conventional circumstances at a temperature of 18-24ºC, with a light/dark cycle of 10/14 hours respectively, and they were provided withfood and water *ad libitum*. Animals were permitted to adapt to the new environment for 30 minutes before the beginning of the experimental. The Ethics committee of the Veterinary Medicine College, University of Duhok approved this research and the use of the experimental rats model.

Experiments

1- Assessment of the median lethal dose (LD50) of ketorolac (I.M) by the up-down method:

This experiment had been done according to the dixon method (19). Seven male rats were used in this experiment.The dose of ketorolac (Darnista, Ukraine 30 mg/ml,vial) was 500 mg/kg administered IM injection, based on a pilot study. The response of rats to ketorolac was evaluated as either stay alive or dead (O toward alive and X toward dead) for each animal 24 hours post-injection. The onset of acute toxicity signs and mortality was documented. The variation in Ketorolac dosage was maintained at a constant value of 125 mg/kg. By calibrating this dosage in both the upward and downward order among multiple rats, we were able to determine the LD_{50} of Ketorolac using the following equation:
LD50=Xf+Kd ,
where the symbols are analogous to the following:
LD50: Median lethal dosage
Xf: Final administered dose
K: A constant obtained from the Dixon table, d: the constant dosage range (both up and down) is derived.

2- Determining the median analgesic effective dose (ED50) after i.m injection of ketorolac by using electrical stimulator.

In this experiment, five rats were used. The principle of this test is based on the electrical pain response using an electrostimulator device (Scientific and Research Ltd., United Kingdom). This device is able to stimulate the sensation of rats by electro-stimulation process (20) The presence of a sensation reflect point to pain perception in rats (21). Ketorolac was administered intramuscularly to the rats at 20 mg/kg, depending on 1^{st} experiment. The dose was then adjusted using the up-and-down method by increments or decrements of 5 mg/kg, depending on the presence or absence of analgesia. After 30 minutes, the pain response was re-examined using the electrical stimulator. An increase in response time indicates the onset of the analgesic effect, and the rat is marked with the symbol (X); if there is no increase, the rat is marked with the symbol (O). After completing the first and second experiments, the safety indices of ketorolac were assessed using the following equation to evaluate and extrapolate its safety profile depending on the calculated ED50 and LD50 values:

Therapeutic Index (TI) = LD_{50} / ED_{50}
A greater TI value indicates a greater margin of safety for the medicine

3- The analgesic activity of ketorolac against the visceral pain model-writhing test

Twenty-eight rats were divided into 4 groups. The first group administered 5 ml/kg of normal saline by intramuscular injection. The second, third, and fourth groups received ketorolac 20,40, and 80 mg/kg (I.M), respectively. One hour post-dosing, all groups received an intraperitoneal injection of acetic acid (1%) at a volume of 0.1 ml. The onset and frequency of writhing reactions throughout a 30-minute interval were recorded (22)

Statistical analysis :IBM SPSS Statistics (Version 27) was used to analyse the data. Data expressed as mean ± standard error (mean ± SE) was shown for each outcome. One-way analysis

of variance (ANOVA) was employed to determine significant variance among the groups, followed by Duncan's multiple comparisons at a probability level of ($P \leq 0.05$) (23)

Results

1-Determinating (LD50) of ketorolac I.M injection by the up-and-down method

The median lethal dose was 693 mg/kg,i.m , B.W, where the rats displayed signs of acute toxicity within 10 to 15 minutes after administration. The symptoms were lethargy, increased breathing, and Straub's tail, leading to nervous tremors and death. (Table 1).

2-Determining the median analgesic effective dose (ED50) after i.m injection of ketorolac by using an electrical stimulator.

The principle of this test depends on the electrical pain response, which was generated by using an electrical stimulator.The acute median analgesic dosage was 14.39 mg/kg body weight, as shown in Table 2. A therapeutic index of 48indicated that the median effective dosage must be doubled 48 times to achieve a 50% mortality rate in rats.

Table 1: The median lethal dose (LD_{50}) of ketorolac in rats after intramuscular injection using the upward and downward method

Variables	Result
Median lethal dose (LD_{50})	693mg/kg
Dose range	875-500mg/kg
First dose	500mg/kg
Last dose	500mg/kg
Up and down value	125mg/kg
Number of rats	7 (OOOXXXO)
Onset of action	10-15 minutes
Toxicological signs	lethargy, elevated respiration, Straub's tail, resulting in nervous convulsions and death

X: Death of the animal during 24 hours. O: Surviving of the animal during 24 hours.

3- The analgesic activity of ketorolac against the visceral pain model-writhing method

Ketorolac was injected (20,40, and 80 mg/kg) into each rat/group I.M, then after 1 hr Acetic acid 1% (0.1ml) I.P was injected, onset and number of writhing were recorded for 30 minutes. The onset of action at 80mg/kg (5.1 ± 0.49 min) and 40 mg\kg (4.8 ± 0.45 min) significantly increased compared to the control group (2.6±0.18 min), but the dose 20 mg/kg (3.1 ± 0.48 min) **did** not induce a significant differences compared with the control group (Table 3) . All groups showed a

significantly difference in the number of writhes compared to the control group that received normal saline (103 ± 4.63). Number of writhing faded significantly at 40 mg/kg of ketorolac (59.8 ± 1.82) compared to the 20 mg/kg (81.6 ± 1.94), but the dose 80 mg/kg (39.4 ± 1.77) induce a significantly analgescic compared with 20 ,and 40 mg\kg (981.6 ± 1.94 and 59.8 ± 1.82) respectively. The analgesic efficacy percentages of 20,40 and 80 mg\kg were 21, 42, and 62% respectively. (Table 3).

Table 2: Acute median effective dose ED_{50} in male rats after intramuscular injection of Ketorolac

Variables	Result
Median Effective dose (ED_{50})	14.39mg/kg
Dose range	20-10 mg\kg
First dose	20 mg\kg
Last dose	10 mg\kg
Up and down value	5 mg\kg
Number of rats	5 (XOXXO)

X:indicates analgesia O: indicates no analgesia

Table 3: Analgesic activity of ketorolac against visceral pain induce-writhing method

Group	Onset of writhing(min.)	Numbers of writhing	Percentage of Analgesic efficacy (%)
Control (N.S)	2.6 ± 0.18	103 ± 4.63	0
Ketorolac. 20 mg\kg	3.1 ± 0.48	81.6 ± 1.94 *****	21
Ketorolac. 40 mg\kg	4.8 ± 0.45 *****	59.8 ± 1.82 ***a**	42
Ketorolac. 80 mg\kg	5.1 ± 0.49 *****	39.4 ± 1.77 ***ab**	62

The value represent the mean ± SE of 7 rats \ group. at ($p \leq 0.05$).
*** indicates a significant difference compared with the control group,**
a: indicates a significant difference compared to the group ketorolac at 20 mg/kg,
b: indicate a significant difference compared to the group ketorolac at 40 mg/kg.

Discussion

Ketorolac is a medication that is applicable across several administration routes, exhibiting significant analgesic efficacy as seen by the current research. The analgesic effect of ketorolac has

been thoroughly investigated for the treatment of moderate to severe pain (5). Analgesia was equivalent to or superior to aspirin or acetaminophen (24). In contrast to other NSAIDs, ketorolac is a powerful analgesic with higher water solubility and does not cause tissue irritation. It's delivered by different routes such as intravenous, intramuscular, oral, topical, and rectal (5). Ketorolac is a nonsteroidal anti-inflammatory agent (NSAID) recognised for its analgesic properties (25). Determining the median lethal dose is crucial in drug dosing, since it provides insight into the dosages applicable for experimentation and therapy (26). In this study, the median lethal dose of ketorolac was calculated using the up-and-down Dixon technique, which is recognised as ethically approved because it uses a minimal number of animals compared to other methods (27), the lethal dose of ketorolac in rats was 637 mg/kg. Our findings closely align with those of (5,28), they used dixone method indicated an ED_{50} was 9.1 mg/kg,i.m in chicks and 5.62 mg/kg, in rats when administered by intramuscular injection of ketorolac. In the current study, the analgesic activity of ketorolac was assessed using the acetic-acid writhing method, which measures abdominal muscular contractions resulting from irritation of the serous membrane after an intraperitoneal injection of acetic acid solution (29). The pain-relieving effect of ketorolac in the visceral pain model, measured by the writhing test, is indicated by a notable decrease in the number of writhing movements in subjects treated with ketorolac compared to the control group, which is similar to our findings. Its action leads to temporary and non-selective blocking of cyclooxygenase (COX) enzymes (COX-1 and COX-2). Its activity results in reversible non-selective suppression of cyclooxygenase (COX) enzymes (COX-1 and COX-2) (8, 31,32).

Conclusion

The study concludes that ketorolac exhibits a wide therapeutic window in the rat model, indicated by the substantial range between its therapeutic and lethal doses. Furthermore, ketorolac demonstrated peripheral and visceral analgesic activity, confirming its primary mechanism of action via inhibition of prostaglandin synthesis in peripheral tissues and internal organs rather than central nervous system mediation. This suggests the potential for safe use of ketorolac as an analgesic under veterinary supervision.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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تقييم ألتأثير المسكن للألم للكيتورولاك في الجرذان

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الخلاصة

يوجد طلب كبير في الاونه الاخيرة على مسكنات ألالم ألاكثر أمانًا والغير مسببة للإدمان والتي لها تأيرات جانبية قد تكون محدودة. ولهذا السبب اجريت هذه الدراسه لتحديد التأثيرات المسكنة للكيتورولاك في نموذج ذكور الجرذان لأمكانية استخدامه كمسكن في المجالات العملية للطب البيطري. كان متوسط الجرعة المميتة (LD_{50}) للكيتورولاك، والتي أدت إلى نفوق 50٪ من الحيوانات (693 ملغم/كغم) عن طريق الحقن العضل واظهرت الجرذان علامات التسمم الحاد حادٍ (خمول، وزيادة معدل التنفس، وظاهرة شد الذيل) بعد 10-15 دقيقة من اعطاء الدواء. وفي المقابل، كان متوسط الجرعة المسكنة (ED_{50}) التي أدت الى تسكين 50٪ من الحيوانات (14.39 ملغم/كغم) عن طريق الحقن العضلي . كما اثبتت الدراسه بامكتلاك الكيتورولاك تأثيرا مسكنا في اختبار التلوي الناجم عن استخدام حمض الأسيتيك (1%) في غشاء الصفاق حيث أظهرت الجرذان التي أعطيت الكيتورولاك بجرعات متعددة (20 و40 و80 ملغم/كغم في العضلة) انخفاضًا كبيرًا في وتيرة نوبات الالتواء مقارنةً بمجموعة السيطرة التي تلقت المحلول الملحي الفسلجي فقط , حيث كان معدل انخفاض التلوي معتمدا على جرعة الكترولاك ، مما تشير هذه الدراسة إلى التأثير المسكن للألم لعقار الكيتورولاك اعتمادا على الجرعة المعطاة, وبلغت نسبة التسكين من الالم 21%، 42%، و62% على التوالي وحسب الجرع المعطاة. يمكن ان نستنتج من الدراسة ان الكيتورولاك له نافذة امان علاجية واسعة حيث ان المدى بين الجرعة العلاجية والسامة كبير نسبياً مما يدل على إمكانية استعماله بامان وتحت اشراف الطبيب البطري .

الكلمات المفتاحية: كيتورولاك؛ مسكن , حامض الخليك , الجرذان.