



Analgesic and Anti-inflammatory Effects of Montelukast in Chicks

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Abstract

THE GOAL of the study was to assess the analgesic and anti-inflammatory properties of montelukast in chicks. Methods: The oral median analgesic effective dose (ED₅₀) of montelukast was determined by the up-and-down method. An electrical stimulator was used to determine the analgesia of montelukast over time. Formalin test was used to determine the analgesic and anti-inflammatory activities of montelukast. Results: oral median analgesic effective dose (ED₅₀) of montelukast was 9.23 mg/kg. Montelukast (18 and 36 mg/kg) had significant analgesic activity over time compared with a control group, and the peak of analgesia of montelukast was observed later one hour of treatment. Montelukast showed significant analgesic and anti-inflammatory activities in the formalin test, represented by a significant increase in the commencement of lifting the right foot, a decrease in the number of lifting the right foot, and significant a decrease in the thickness of the paw in comparison to control group. Conclusion: Montelukast possesses noteworthy effects of eliminating pain and inflammation in chicks.

Keywords: Montelukast, Analgesic, Anti-inflammatory, Chicks.

Introduction

Arguably the most prevalent sign of many illnesses is pain, which also has an economic impact on the whole world [1]. For this reason, researching new analgesic action of some drugs is crucial to improving the lives of patients [2]. Current pain-relieving drugs like opioids and non-steroidal anti-inflammatory drugs are excellent at relieving pain but they have serious side effects such as damage to the kidneys, vomiting, breathing disorders, gastrointestinal problems, tolerance, and addiction [3].

A family of powerful eicosanoid lipid mediators derived from the metabolism of arachidonic acid e.g. leukotrienes, it has a wide range of biological actions with a potent effect on inflammation and immunity. When a cell is activated, they are made from membrane phospholipids [4]. Arachidonic acid converts through the 5-lipoxygenase (5-LOX) pathway to cysteine-leukotrienes (CysLTs), which bind to the CysLT1 and CysLT2 receptors [5]. The

principal pharmacodynamic of montelukast is the block of cysteinyl leukotriene (cys-LT) receptors in the lungs which reduces inflammatory processes and relaxes smooth muscle [6]. When used to treat asthma, montelukast has been shown to lessen eosinophilic inflammation in the respiratory tract [7]. Montelukast is prescribed to treat urticarial and allergic rhinitis[8].

Recent preclinical research has demonstrated that antinociception was elicited in various inflammatory pain models by zileuton's suppression of the lipoxygenase (LOX) enzyme or by the antagonistic action of zafirlukast and montelukast on leukotriene receptors [9].

Outstanding the novelty of the topic and the scarcity of studies in laboratory animals, as well as the lack of a study on the chicken model in the field of veterinary medicine. This study uses an electrical stimulator and formalin test to examine the analgesic and anti-inflammatory activity of montelukast in chicks.

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Material and Methods

Ethical agreement

College of Veterinary Medicine Ethics Committee Standards Rules were followed when managing the animals. This work was authorized by the Scientific Council of Physiology, Biochemistry, and Pharmacology Branch/Veterinary Medicine College/ University of Mosul (Protocol ref: UM. VET. 2022. 054).

Animals and drugs

Experiments were carried out using Ross chicks (one-day old of both sexes, total number:41) brought from a dependable hatchery and bred in the college field until the experiments were finished in (7-10) days. Chicks were housed in cages at 32-35°C with consistent lighting, mulch on the floor, and free access to water and drug free food [10,11].

Montelukast (4 mg/tablet, Pharma International Co. Amman, Jordan) was dissolved in a saline solution to obtain the required drug concentrations. The montelukast was given orally at a volume of administration 5 ml/kg.

Experiments

Determine the oral median analgesic effective dose (ED₅₀) of Montelukast.

Up-and-down method [12] was used to estimate the median effective analgesic dose of montelukast administered to chicks orally. The electrical stimulator device (SRI, England) was programmed with the following parameters: frequency, 50 Hz; width, 5 Hz; and amplitude, 10 volts. The electrical stimulator rods were prudently sited beneath the wings of chicks in a featherless region and gently increased in voltage until the wings of chicks fluttered as a result of the stimulating pain [13,14]. Both chicks were exposed to a voltage-caused slight pain reaction before and one hour after montelukast treatment. An increase in voltage after treatment indicated an analgesic effect [15].

The analgesic effect of Montelukast over time

Chicks were divided into three groups (6 chicks/group). The chicks in group 1 received normal saline, chicks in groups 2 and 3 were received montelukast 18 and 36 mg/kg orally, respectively (the dose of montelukast was the analgesic ED₁₀₀ and ED₂₀₀ from the earlier trial). The number of voltages that induced a pain reaction in all chicks were recorded at 0, 1, 2, 3, and 4 hrs post-treatment. The record voltages were statistically analyzed in each group to determine the analgesic responsiveness of chicks to montelukast.

Formalin test

Formalin test [16] was used to evaluate the activity of montelukast against pain and inflammation induced by formalin injection in the paw of chicks. Chicks were divided into three groups (6 chicks/group). Montelukast was given orally to the groups at dosages of 0 (control), 16, and 38 mg/kg, respectively. Subsequently 15 minutes, 0.1% formalin (0.05 ml) was injected into the paw of right foot to recruit the inflammation reactions. The paw of left foot received a 0.05ml injection of normal saline as a control. The onset and number of right foot lifts were recorded within 5 minutes following injection of formalin to assess analgesic efficacy. Furthermore, we evaluated anti-inflammatory efficacy by measuring foot thickness (millimeter) formerly and one hour later of the formalin injection with a digital caliper [17]. The anti-inflammatory reaction was determined as follows (in percentages):

$$\text{The anti-inflammatory activity \%} = \frac{[V_{\text{control}} - V_{\text{test}}]}{V_{\text{control}}} \times 100$$

Statistical analysis

All results are displayed as Mean±SEM for six animals in each group. To compare treatment groups, a one-way ANOVA was employed followed by the LSD post hoc test.

Results

The median analgesic effective dose (ED₅₀) of montelukast determined via up-and down method in chicks was 9.23 mg/kg, p.o. (table 1).

Montelukast treatment at 18 and 36 mg/kg, p.o. demonstrated a dose-dependent analgesic effect in compare with control group (table 2). Maximal analgesic effect of montelukast was noted after one hour of therapy and gradually dropped over time to stop in the fourth hour of treatment in the group treated with 18 mg/kg, whereas analgesia continued longer in the group given with 36 mg/kg (figure 1).

In formalin test, montelukast at 18 and 36 mg/kg p.o. elicited analgesia counter to the pain caused through injection of formalin into the paw of the chicks. This was demonstrated by a significant increase in the commencement of lifting the right foot and decrease in the number of lifting the right foot in comparison with control group (table 3).

The anti-inflammatory activity was seen in a substantial decrease in paw thickness in compare with control group. The percentages of anti-inflammatory activity were 42% and 65%, separately, in compare with control group (table 3).

TABLE 1. Median analgesic effective dose of montelukast.

Variable	Result
ED ₅₀ (orally)	9.23 mg/kg
Range of dose	7.5-12.5 (mg/kg)
First dose	10 (mg/kg)
Last dose	10 (mg/kg)
Fixed decrease or increase in dose	2.5 (mg/kg)
Number of chicks, signs, and doses	5, (xooxx) ^a , (10,7.5,10,12.5,10)
Equation application	ED ₅₀ =Xf + Kd ED ₅₀ =10+(-0.305)2.5= 9.23

^aX= analgesia ; O= no analgesia, Up-and-down method after 1 hour of oral administration.

Xf: last dose administered in the trial

K: Tabular value

d: fixed decrease and increase in dose

TABLE 2. Analgesic effect of montelukast (18 and 36 mg/kg) with time in chicks.

Groups	Voltage caused pain with time (hour)				
	0	1	2	3	4
Control	8.31±0.14 ^a	8.49±0.17 ^a	8.40±0.17 ^a	8.36±0.19 ^a	8.36±0.13 ^a
Montelukast 18 mg/kg	8.19±0.15 ^d	12.47±0.17 ^a	11.51±0.19 ^b	9.77±0.25 ^c	8.24±0.14 ^d
Montelukast 36 mg/kg	8.73±0.24 ^e	13.97±0.19 ^a	13.11±0.14 ^b	11.92±0.09 ^c	10.89±0.29 ^d

Mean±SE (6 chicks/group).

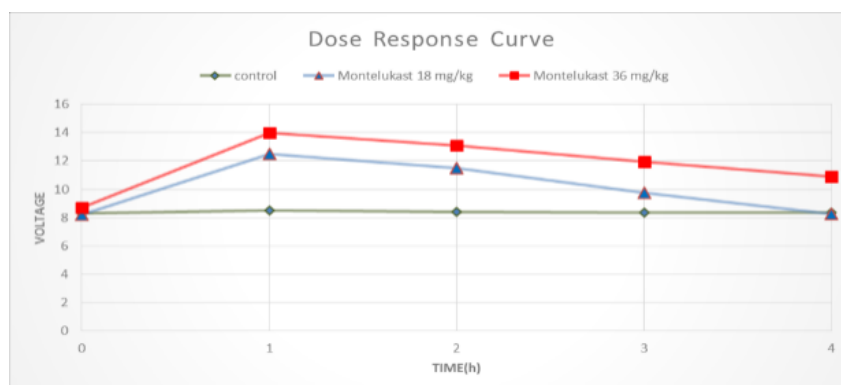
Values in each column followed by different superscript letters are significantly different ($p \leq 0.05$).

TABLE 3. Analgesic and anti-inflammatory activity of montelukast (18 and 36 mg/kg) in formalin test.

Groups	Commencement of lifting right foot (second)	Number of lifting right foot (5 min)	Thickness of paw (mm)	Anti-inflammatory protection %
Control	1.20±0.26 ^a	65±2.17 ^a	0.81±0.09 ^a	-
Montelukast 18 mg/kg	3.36±0.45 ^b	31±1.89 ^b	0.47±0.05 ^b	42
Montelukast 36 mg/kg	3.89±0.57 ^b	33±1.25 ^b	0.28±0.04 ^c	65

Mean±SE (6 chicks/group).

Values in each column followed by different superscript letters are significantly different ($p \leq 0.05$).

**Fig. 1. Dose response curve of montelukast analgesia over time.**

Discussion

In our study, montelukast has a significant analgesic action for acute pain (electro-stimulation test) and anti-inflammatory activity represented by decreasing inflammation and pain (formalin test) in chicks. Chicks served as a model for assessing pain [18,19,20]. Holloway observed that nociceptors in chickens and mammals are physiologically compared, the receptive field size and discharge patterns are quite similar [21]. Based on that the analgesic effects of pharmaceutical substances in chickens can provide a comparable understanding of how analgesics work in mammals.

Determining the median analgesic effective dose (ED50) value is crucial for determining the doses used in following experiments and serves as an indicator of how well a medicine works in laboratory animals utilized in studies [22].

Significant discomfort and vocalization are brought on by the electric shock, and these behaviors eventually lead to violent escaping represented by skipping and flailing wings in an attempt to flee [23]. An electrical stimulator was employed to temporarily cause acute local pain (electric prick). Based on this test, the analgesic effect of various drugs can be determined. The median analgesic effective dose for montelukast was 9.23 mg/kg. Based on this dose, the analgesic effect of montelukast was studied over time. It was observed that the highest analgesia occurred one hour after administration, then it decreases and ended after four hours. So, this indicates the short analgesia time of montelukast.

The investigator noted that while montelukast (20 mg/kg) was unsuccessful in the tail flick test, it exhibited substantial analgesic efficacy when compared with control in the acetic acid-induced writhing test [24]. According to a study by Jain *et al.*, the cysteinyl LT receptor antagonist zafirlukast reduced the inflammatory and nociceptive responses in rats and mice [25].

Several studies in humans have also shown an analgesic effect of montelukast in some clinical condition like dysmenorrhea [26], cystitis [27], migraine [28], tonsillectomy [29], sickle cell disease [30], neuro-pathic pain [31].

There is proof that locally produced leukotrienes function to make peripheral nociceptors more sensitive to painful stimuli. This, in turn, causes the spinal cord to release additional mediators, which causes hyperalgesia [32]. Based on that the mechanism of analgesic action can be attributed to the blocking of cysteinyl leukotriene receptors in the spinal cord thus disabling pain reflexes from reaching higher centers. Another study has indicated that opioid receptors in both the central and peripheral nervous

systems mediated the mechanism of the antinociception effects of montelukast [33].

Toxic, immunological, and microbiological agents can all cause an inflammatory response by triggering different cellular and humoral mediators [34]. During inflammation, membrane phospholipids produce arachidonic acid, which is the precursor to pro-inflammatory eicosanoids. Then Cyclooxygenase and lipoxygenase pathways convert arachidonic acid into prostaglandins and leukotrienes, separately [35].

Formalin was injected intraplantarly to induce a biphasic response, and dissimilar analgesics can react inversely in each of the two phases. The initial reaction is linked to the direct and acute stimulation of noci-captive C-fibers, while the subsequent stage is associated with the discharge of intrinsic mediators in the area. These mediators are in charge of sensitizing primary and spinal sensory neurons, which in turn triggers the activation of nociceptors [36].

In our study, the analgesic effect of montelukast was observed in both phases of formalin test. The montelukast affects the two phases of the formalin test indicates that it has central and peripheral antinociception actions may contribute to its analgesic activity [33].

Investigation has demonstrated that leukotrienes lower the activation threshold of C-nociceptors and increase the sensitivity of intrapulpal A-delta fibers in the hind limbs of rats. When inflammatory stimuli cause the formation of leukotrienes, montelukast suppresses their effects by blocking the cysteinyl LT receptors[37]. Unlike the traditional antagonistic effects of CysLT receptors, montelukast may have anti-inflammatory qualities such as inhibiting P2Y receptors, promoting sustained production of interleukin-10, or interfering with nuclear factor kappa B activation in inflammatory cells[38].

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that are expressed in peripheral nervous system neurons and immune cells. They have a major impact on pain and inflammatory diseases [39]. Alizamani *et al.*, reported the montelukast inhibit peripheral PPAR γ receptors and exert the role in the analgesic action[37].

In conclusion, the results revealed that montelukast has a significant analgesic action for acute pain and markedly anti-inflammatory activity. We also recommend conducting further studies to ensure its safe use from a clinical standpoint

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None

Conflict of Interest

The authors declared no potential conflicts of interest

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التأثيرات المسكنة والضادة للالتهابات للمونتيلوكاست في افراخ الدجاج

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فرع الفسلجة والكيمياء الحياتية والأدوية - كلية الطب البيطري - جامعة الموصل – الموصل - العراق.

كان الهدف من الدراسة هو تقييم الخصائص المسكنة والضادة للالتهابات للمونتيلوكاست في افراخ الدجاج. طرق العمل: تحديد الجرعة الفعالة المتوسطة المسكنة عن طريق الفم للمونتيلوكاست بطريقة الصعود والنزول. استخدام جهاز المحفز الكهربائي لتحديد تسكين المونتيلوكاست مع مرور الوقت. استخدام اختبار الفورمالين لتحديد التأثير المسكن والضاد للالتهابات للمونتيلوكاست. النتائج: متوسط الجرعة الفعالة المسكنة عن طريق الفم للمونتيلوكاست كانت 9.23 ملغم/كغم. كان للمونتيلوكاست بالجرع (18 و 36 ملغم/كغم) نشاط مسكن معنوي مع مرور الوقت مقارنة بمجموعة السيطرة، ولوحظت ذروة التسكين للمونتيلوكاست بعد ساعة واحدة من العلاج. أظهر المونتيلوكاست نشاطاً مسكناً ومضاداً للالتهابات في اختبار الفورمالين إذ تمثل في زيادة معنوية في بدء رفع القدم اليمنى وانخفاض في عدد مرات رفع القدم اليمنى وانخفاض في سمك القدم مقارنة مع مجموعة السيطرة. الاستنتاج: يمتلك المونتيلوكاست تأثيرات ملحوظة في القضاء على الألم والالتهابات في افراخ الدجاج.

الكلمة المفتاحية: مونتيلوكاست، تسكين، ضاد للالتهابات، افراخ الدجاج.