



Evaluation of Pain and Inflammation Protection Activities of Meloxicam in Chickens



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Abdullah D. Abdullah¹, Khalid A. Hadi¹ and Yasser M. Albadrany²

¹Department of Pharmacology, Physiology, and Biochemistry, College of Veterinary Medicine, University of Tikrit, Tikrit, Iraq.

²Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

THIS study aimed to explore the pain and inflammation protective effects of meloxicam in chickens. **Methods:** The median lethal dose (LD_{50}) and median effective analgesic dose (ED_{50}) of intraperitoneally (i.p.) administered meloxicam were determined using an up-and-down technique. Drug safety indices based on the collected results. The dose-dependent analgesic efficacy of meloxicam in chicks was determined by electrical stimulation. The formalin test was used to validate the pain and inflammation protective properties. **Results:** The median lethal dose (LD_{50}) was 156.5 mg/kg intraperitoneally. The median effective analgesic dose (ED_{50}) of meloxicam in chicks was 8.25 mg/kg intraperitoneally. Meloxicam's therapeutic index, standard safety margin, and therapeutic ratio when administered intraperitoneally, were 20, 0.4, and 6.7, respectively. Meloxicam's dose-dependent analgesic effect at 8 mg/kg and 16 mg/kg ip began 0.5 h after treatment and persisted for more than 4 hours. The analgesic effect of meloxicam peaked 2 h after intraperitoneal administration. Meloxicam induced a substantial increase in the latency to raise the right foot in the formalin test when compared to the control value, as well as a significant decrease in foot lifting frequency. The foot thickness decreased significantly compared to the control value. **Conclusion:** These findings indicate that meloxicam has pain and inflammation protective properties, which will serve as the foundation for future pharmacological investigations, and that this medicine may be safely administered to chickens.

Keyword: Meloxicam, Pain, Inflammation, Protective, LD_{50} , ED_{50} , Chickens.

Introduction

Pain is an unpleasant sensory experience that is associated with actual or potential tissue damage. Pain measurement techniques that are both effective and accurate must be developed[1]. Although analgesia is an important part of veterinary medicine, the identification of pain in avian species remains difficult because of species-specific pain manifestations[2]. The issue of pain management in animals is similar to that in human pediatrics in that the patient is nonverbal and the

doctor must rely on personal observations and reports from the patient's advocate. As a result, doctors have long wrestled with criticism of undermanaging pain in youngsters [3].

Nonsteroidal anti-inflammatory medications (NSAIDs) are among the most powerful and effective analgesics used for the treatment of both acute and chronic pain [4]. Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) with pain-relieving, anti-inflammatory, and antipyretic properties. It acts by non-selectively

blocking the cyclooxygenase enzymes (COX1 and COX2 isoforms), which lowers prostaglandin synthesis[5].

The aim of the study was to determine the pain and inflammation protective effects and safety profile of a meloxicam in chicks.

Material and Methods

Ethical approval

The animal ethics committee guidelines of the College of Veterinary Medicine were followed for handling the birds. This study was approved by the scientific board of the Department of Physiology, Biochemistry, and Pharmacology, Faculty of Veterinary Medicine, University of Tikrit (**Protocol no. 3/7/1215**).

Experimental animals and drug

Forty eight one-day-old Ross chicks that were purchased from a licensed hatchery and kept until the tests were done in seven–nine days. Chicks were kept in a chamber at a temperature between 32 °C and 35 °C, with constant lighting, sawdust on the floor, and constant access to food and water. Meloxicam (5mg/ml, Ashish Life Science Pvt limited, India) was extra diluted in saline solution (Pioneer Company for Pharmaceutical Industries, IRAQ) to gain the necessary drug concentrations. The volume of drug administration was 5 ml/ kg body weight given intraperitoneally (i.p.).

Experiments

Determination of intraperitoneal median lethal dose (LD50) of meloxicam.

Acute (24 h) LD₅₀ was determined using the up-and-down method[6] following intraperitoneal administration. The chicks were individually assessed for clinical symptoms of toxicity two hours after receiving meloxicam. The lethality was recorded for 24 h.

Determination of the intraperitoneal median effective dose (ED50) of meloxicam for the induction of analgesia in chicks

The pain protective effect of meloxicam administered to the chicks was evaluated using the up-and-down method. The setting of the electrical stimulator (SRI, Science and Research Instruments, United Kingdom) was adjusted according to a frequency of 50 Hz, width of 5 Hz, and pulse amplitude of 10 volts. The stimulator electrodes were carefully placed under the wing in a featherless area that had been moistened with distilled water. The chicks flapped their wings in response to an electrical stimulation device which

generated pain [7]. Each chick was subjected to a voltage that elicited an unpleasant pain response before and 30 minutes after treatment (triggered pain voltages were recorded before and after treatment). Each chick was tested to determine whether there was an increase or not in voltage that caused a pain response. In general, a positive analgesic response delay was observed 2s after electrical stimulation.

Determination of drug safety indices

The drug safety indices for meloxicam were calculated from the results of prior experiments using the following formula: Therapeutic Index (TI) =LD₅₀/ED₅₀, Standard Safety Margin (SEM) =LD1/ED99, and Therapeutic Ratio (TR) = LD25/ ED75[8].

The pain protective of meloxicam with time

Eighteen chicks were randomly allocated to three groups of six birds. The chicks were administered normal saline (control) or meloxicam (8 and 16 mg/kg bw, i.p.). The meloxicam dose used was the analgesic ED₅₀ and ED₁₀₀ (grounded in a previous experiment). We assessed the voltage that elicited an unpleasant pain response in each chick at 0, 0.5, 1, 2, and 4 hrs after treatment. The increase in voltage was statistically assessed in each group to measure the analgesic reaction of the chicks to meloxicam.

Formalin test to determine meloxicam protective effect on pain and inflammation in chickens.

The pain and inflammation protective properties of meloxicam were assessed using the formalin test. Eighteen chicks were allocated to three groups of six birds at random. The three groups of chicks were administered meloxicam intraperitoneally at doses of 0 (control), 8, and 16 mg/kg, respectively. Fifteen-minute after treatment, the chicks were injected in the right foot plantar with 0.1 % formalin (0.05 ml) to initiate the pain and inflammatory reactions. The left plantar foot was injected with normal saline (0.05 ml) as a control. The onset of right foot raising and the number of raising the right foot were recorded within 3 min of formalin injection for determination of pain protective efficacy. In addition, we assessed inflammation protective activity of meloxicam by measuring foot thickness (mm) using a digital caliper (Electronics Lab, China) before and one hour after formalin injection[9]. The anti-inflammatory reaction was measured as following (percentage):

The inflammation protective response % = [alteration in control group foot thickness – alteration in treatment group foot thickness / alteration in control group foot thickness] ×100

Statistical Analysis

Data has been described as mean ± standard errors. Statistical analysis was carried out by using one-way analysis of variance (ANOVA) and then subjected to LSD test. P<0.05 were considered to be significant. The measurements were conducted using the statistical software SPSS 17.

Results

The acute LD₅₀ (24 h) of meloxicam administered intraperitoneally in chickens was 156.5 mg/kg (Table 1). Anxiety, screaming, apnea, wing drooping, dullness, shrunken eyes, and recumbency are the symptoms of acute poisoning. The intraperitoneal ED₅₀ value of meloxicam for inducing analgesia in the chicks was 8.25 mg/kg i.p. (Table 2). The therapeutic index (TI),

standard safety margin, and therapeutic ratio of intraperitoneally administered meloxicam were 20, 0.4 and 6.7, respectively.

Meloxicam administration at 8 and 16 mg/kg i.p. produced a dose-dependent analgesic effect with time compared to the control group, which was treated with normal saline only. The analgesic effect in the treatment groups began 30 min after administration and lasted over 4 h (Table 3), with the peak at 2h (Figure 1).

Meloxicam at 8 and 16 mg/kg i.p. provided analgesia against the pain generated by formalin injection into the plantar area of the chick's foot in the formalin test. This was demonstrated by a considerable increase in the onset of right foot raise and a significant decrease in the number of right foot raises relative to the control value (Table 4). The anti-inflammatory action of meloxicam resulted in a significant decrease in foot thickness compared with the control value. Compared with the control group, the proportions

TABLE 1. Median lethal dose (LD₅₀) of Meloxicam in chicks by the up-and-down method after 24 h.

Variable	Result
LD ₅₀ (mg/kg) (ip)	156.5
Doses range (mg/kg)	150-200
Early dose(mg/kg)	200
Latest dose(mg/kg)	175
Increase or decrease in dose(mg/kg)	25
Total of chicks used, Symbols and their corresponding dose	6 (xxoxox) ^a (200-175-150-175-150-175)
Equation application	LD ₅₀ =Xf + Kd LD ₅₀ =175+(-0.737)25=156.575

^aX- death ; O- live

TABLE 2. Median effective dose (ED₅₀) of Meloxicam in chicks by the up-and-down method after 30 min.

Variable	Result
ED ₅₀ (mg/kg) (ip)	8.25
Doses range (mg/kg)	7.5-10
Early dose(mg/kg)	10
Latest dose(mg/kg)	10
Increase or decrease in dose(mg/kg)	2.5
Total of chicks used, Symbols and their corresponding dose	6 (xoxox) ^a (10-7.5-10-7.5-10)
Equation application	ED ₅₀ =Xf + Kd ED ₅₀ =10+(-0.701)2.5= 8.25

^aX- analgesia ; O-no analgesia

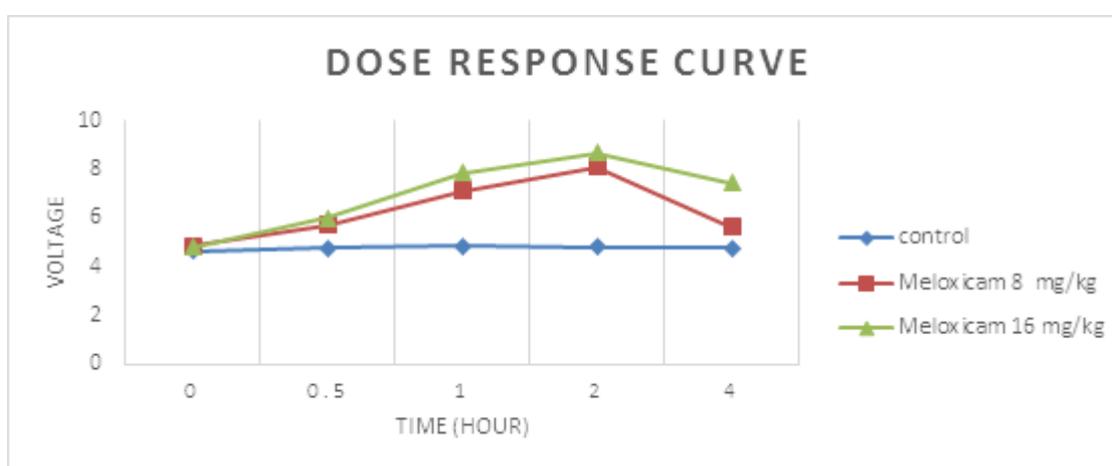
TABLE 3. Effect of Meloxicam different doses (8 and 16 mg/kg bw) on pain protection over time in chickens.

Groups	Voltage caused pain after				
	Time hour				
	0	0.5	1	2	4
Control	4.67±0.25 ^{aA}	4.78±0.16 ^{aA}	4.86±0.22 ^{aA}	4.82±0.30 ^{aA}	4.80±0.19 ^{aA}
Meloxicam 8 mg/kg	4.86±0.16 ^{aA}	5.73±0.23 ^{bB}	7.11±0.28 ^{cB}	8.11±0.12 ^{dB}	5.63±0.10 ^{bB}
Meloxicam 16 mg/kg	4.81±0.20 ^{aA}	6.00±0.21 ^{bB}	7.88±0.10 ^{cB}	8.68±0.08 ^{dB}	7.47±0.13 ^{cC}

Values represent mean± SE for 6 chicks/group.

At the 5 percent significance level, the values of each row followed by different small letters are significantly different.

At the 5 percent significance level, the values of each column followed by different capital letters are significantly different.

**Fig. 1. Dose response curve of meloxicam.**

of anti-inflammatory activity were 51% and 58%, respectively (Table 4).

Discussion

The measurement of acute toxicity is often performed at an early screening stage during the examination and evaluation of the hazardous characteristics of all substances [10]. Acute toxicity includes the determination of the LD₅₀ (the dose that proved to be deadly to 50% of the animal group tested). In our study, the median lethal dose for chicks was 156.5 mg/kg i.p., and the toxicological signs observed were anxiety, screaming, apnea, wing drooping, dullness, shrunken eyes, and recumbency. In previous studies, The oral LD₅₀ in rats was 84 mg/kg, and animals administered meloxicam displayed weakness, lethargy, and bloated belly [11]. The oral LD₅₀ in mice was 343.04 mg/kg, and animals administered meloxicam displayed restlessness or nervousness, elevated tail, grooming with fast-
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breathing tremors, convulsions, laying down on one side, and death occurred during the course of 24 h of monitoring [12].

Determination of the ED₅₀ value is an indication of the action of the drug in the laboratory animals used in experiments in experimental and preclinic studies, as it is critical in determining the doses used in the clinic. The electric shock causes considerable discomfort and vocalization, culminating in aggressive avoidance behaviors such as escape attempts (jumping and wing flapping) [13]. An electrical stimulator was used to induce local pain for a short duration (electric prick). The median effective dose of meloxicam to induce analgesia was 8.25 mg/kg and which was calculated using the up-and-down method. Based on the LD₅₀ and ED₅₀ values, we determined the therapeutic index, standard safety margin, and therapeutic ratio of intraperitoneally administered meloxicam. From the results we obtained, there

TABLE 4. Meloxicam pain and inflammation protection parameters in chickens.

Groups	Onset of raising right Foot (second)	Number of raising right foot (within 3min)	The increase in paw thickness (mm)	Inflammation protection %
Control	1.50±0.34 ^a	34.00±1.65 ^a	0.87±0.12 ^a	0
Meloxicam 8 mg/kg ip.	2.50±0.42 ^a	24.50±0.76 ^b	0.31±0.03 ^b	51
Meloxicam 16mg/kg ip.	4.00±0.57 ^b	22.33±1.30 ^b	0.25±0.03 ^b	58

Values represent mean±SE for 6 chicks/group.

At the 5 percent significance level, the values of each column followed by different small letters are significantly different.

is a high level of safety when using meloxicam in chickens through intraperitoneal routes, up to twenty fold. Many NSAIDs may have additional mechanisms that interact with the monoaminergic, nitric oxide, endocannabinoids, serotonergic, cholinergic, and endogenous opioid pathways [14]. This suggests that meloxicam acts to mitigate acute pain caused by electrical stimulation.

Our findings indicate that the peak analgesic effect of meloxicam was observed 2 h after intraperitoneal injection (ED_{50} and ED_{100}), which was initiated rapidly after 0.5 hours and lasted over 4 hours. From the concentration curve over time, we noted the speed of action of meloxicam as an analgesic at specified doses and the continuation of its analgesic effect for more than four hours, which enables its clinical use.

In the formalin test, the analgesic and anti-inflammatory effects of meloxicam were detected, which was evident in the increase in the onset of raising the right foot and decrease in the number of raising the right foot, also the anti-inflammatory action was evident in the decrease in foot thickness.

Formalin injection into the paw generates biphasic nociceptive reflexes. Although phase I represents acute nociceptive pain caused by formalin-induced nerve stimulation, phase II is linked to a mix of inflammation-associated peripheral tissue afferent inputs and functional alterations in the spinal horn (central sensitization) [15]. The transitory initial phase begins with the direct effect of formalin on transient receptor potential ankyrin subtype 1 receptors (TRPA 1). The second protracted phase is accompanied

by an inflammatory reaction in the peripheral tissues. This response causes the production of nociceptive mediators such as serotonin, histamine, bradykinin, and prostaglandins, which cause central neuron sensitization and alterations in pain regulation systems [16]. Inflammation is linked to the release of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and cyclooxygenase-2 (COX-2) [17]. Inflammatory mediators such as PGE2 and bradykinin can sensitize peripheral nociceptors to pain stimuli, resulting in hyperalgesia or increased pain sensitivity [18]. Tumor necrosis factor and IL-1 have been shown to increase COX-2 production [19]. Analgesic and anti-inflammatory roles of meloxicam, an NSAID, via inhibition of IL-1 β and TNF- α , which are involved in the inflammatory process [20]. In addition to the major effects of NSAIDs, cyclooxygenase is blocked, preventing the final conversion of arachidonic acid into prostaglandins, prostacyclin, and thromboxanes [21].

Conclusions

We conclude that meloxicam has analgesic and anti-inflammatory properties in chicks and is safely used for its wide range between the therapeutic and lethal dose, with the advice for more studies to determine a treatment protocol.

Acknowledgment

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Conflict of Interest

There are no conflicts of interest.

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تقييم النشاط الوقائي من الألم والالتهاب للميلوكسيكام في افراخ الدجاج

عبدالله ضياء عبدالله^١ وخالد احمد هادي^١ وياسر محمد امين البدراني^٢

^١ فرع الادوية والفسلجة والكيمياء الحياتية - كلية الطب البيطري - جامعة تكريت - تكريت - العراق.

^٢ فرع الفسلجة والكيمياء الحياتية والأدوية - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.

تهدف الدراسة إلى الكشف عن التأثيرات الوقية من الألم و اللالتهابات للميلوكسيكام في افراخ الدجاج. طرق العمل: تم تحديد الجرعة المميّنة الوسطية (LD50) والجرعة الفعالة المسكنة الوسطية (ED50) للميلوكسيكام عن طريق الحقن داخل الصفاق (i.p.) باستخدام طريقة الصعود والهبوط. ومؤشرات سلامة الدواء بناءً على النتائج التي تم الحصول عليها. تم تحديد الوقاية للألم لجرعتين من الميلوكسيكام في الافراخ عن طريق جهاز التحفيز الكهربائي. واستخدام اختبار الفورمالين للتحقق من قدرة الميلوكسيكام علي الوقاية من الألم والالتهاب. النتائج: كانت الجرعة المميّنة الوسطية ١٥٦,٥ ملغم / كغم داخل الصفاق. وكانت الجرعة الفعالة المسكنة الوسطية ٨,٢٥ ملغم / كغم داخل الصفاق. كان المؤشر العلاجي وهامش الأمان القياسي والنسبة العلاجية (٢٠) و (٠,٤) و (٦,٧) على التوالي. بدأ التأثير الوقائي من الألم بجرعة ٨ و ١٦ ملغم / كجم عند ٠,٥ ساعة بعد الحقن واستمر لأكثر من ٤ ساعات. بلغ التأثير الوقائي من الألم للميلوكسيكام ذروته بعد ساعتين من الحقن داخل الصفاق. تسبب ميلوكسيكام في زيادة معنوية في بداية رفع القدم اليمنى في اختبار الفورمالين عند مقارنتها بالمجموعة الضابطة ، بالإضافة إلى انخفاض معنوي في عدد مرات رفع القدم. انخفض سمك القدم بشكل معنوي مقارنة مع المجموعة الضابطة الخلاصة: تشير النتائج إلى أن الميلوكسيكام له خصائص مبدئية للوقاية من الألم والالتهاب ، والتي تعتبر بمثابة نقطة بداية لدراسات السلامة والدوائية المستقبلية ، وأن هذا الدواء يمكن أن يُعطى بشكل آمن في الدجاج بعد استكمال الدراسات المعملية والحقلية.