Effect of meloxicam coadministration on the anaesthetic potency of thiopental sodium in a chick model

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Abstract

Few studies have dealt with thiopental sodium-induced anaesthetic action and the effect of combining meloxicam (a high plasma protein-bound) in 10–15 day old chicks. First, the analgesic median effective dose ($ED_{50}$) was determined as 35.85 mg/kg, IM by up-and-down routine, while the hypnotic $ED_{50}$ value was 34.40 mg/kg, IM in the chick model. A thiopental sodium injection (18, 36 and 72 mg/kg, IM) produces a significant dose-responsive hypnotic effect in chicks, determined by the beginning of the lack of a righting reflex, duration and recovery time. Thiopental sodium and meloxicam (72 and 1 mg/kg, IM) in combination shortened the beginning of hypnosis, and significantly extended its duration, with a significant increase in recovery time from the hypnotic effect when compared to the group receiving only thiopental sodium. The same combination also elicited a significant increase in the analgesic percentage and efficacy, and significant increase in the voltage current estimated via using electrical stimulation to induce the ache feeling. No significant changes were found in the concentrations of serum glutamate pyruvate trans-aminase (GPT), glutamate oxalo-acetate trans-aminase (GOT) with body temperature between the two groups, with the exception of a significant change in respiratory rate. The outcomes of this study support the prospect of using thiopental sodium as an anaesthetic agent for veterinary surgical procedures in the chicks, in combination with meloxicam, to produce worthy, consistent, and proficient anaesthesia.

Key words: analgesia; chick model; hypnosis; meloxicam; thiopental sodium

Introduction

Thiopental sodium is an ultra-short anaesthetic agent used to quickly induce general anaesthesia. The mechanism by which thiopental sodium induced anaesthesia results in inhibition of the central nervous system (CNS) is by potentiating activity of the gamma-aminobutyric acid (GABA)
neurotransmitter (Finkel et al., 2009). Thiopental sodium has a good hypnotic effect with less efficacy in analgesia in comparison to other narcotic agents (Finkel et al., 2009; Flecknell, 2009; White and Trevor, 2009). Meloxicam belongs to the category of non-steroidal anti-inflammatory agents (NSAIDs) and has an pain-relieving, anti-inflammatory and antipyretic activity that works by non-selectively inhibiting the cyclooxygenase enzyme (both COX1 and COX2 isoforms), and this reduces prostaglandin production (Smyth and FitzGerald, 2009; Hilal-Dandan and Brunton, 2014). Due to its analgesic, anti-inflammatory, and antipyretic effects, meloxicam may be used as pre-anaesthetic (premedication) or in conjugation with anaesthetic drugs for enhancing the anaesthetic status in surgical operations like hypnosis and analgesia. Meloxicam is considered a high plasma protein-bound (Turck et al., 1996) that may affect the efficacy of thiopental sodium.

For the above reasons, the aim of this study was to use and authenticate thiopental sodium to induce general anaesthesia in a chick model for the first time, and to determine the likely effect of meloxicam on the anaesthetic property of thiopental sodium for use in veterinary medical field, and to determine its suitability in replacing the usual anaesthesia protocol in animals using a mixture of ketamine and xylazine.

Materials and methods

Chick model and drugs used

Seven to fifteen-day old broiler chicks of both genders were used in all trials with a regular body weight between 120–178 g. They were kept under constant heat lamps at a temperature of 30–37°C. The floor was covered with wood shavings. All chicks had access to drinking water and feed ad libitum. Thiopental sodium (2.5%, Egyptian International Pharmaceutical Industries Co., Egypt) and meloxicam (2%, Intracin Pharmaceuticals Private Ltd., India) were diluted with saline solution (0.9% NaCl) for injection at 10 ml/kg, intramuscularly (IM).

Ethical considerations

This research and the use of the experimental chick model were approved by the Ethics committee of the Veterinary Medicine College, University of Mosul and its Department of Physiology, Biochemistry, and Pharmacology.

Assessment of thiopental sodium ED$_{50s}$ in the chick model

A. Analgesic ED$_{50}$ of thiopental sodium

The hypnotic ED$_{50}$ value of thiopental sodium was estimated according to the up-and-down routine designated by Dixon (1980). The first dose of thiopental sodium was at 40 mg/kg, IM based on an introductory study. The chick model measured independently prior and 15 minutes after thiopental sodium administration treatment using the electro-stimulator device (Harvard apparatus, USA) (presence of distress call point to pain perception in chicks) (Mousa and Mohammad, 2012; Mousa, 2014; Mousa and Al-Zubaidy, 2019), and then the doses of thiopental sodium were lowered or raised by 5 mg depending on the presence or absence of analgesia (decrease or increase in doses was chosen whereby not exceeding 30% of the first dose of thiopental sodium for an accurate outcome).

B. Hypnotic ED$_{50}$ result of thiopental sodium

The value related to hypnotic ED$_{50}$ for thiopental sodium was estimated using the up-and-down manner outlined above (Dixon, 1980). The first dosage of thiopental sodium was 40 mg/kg, IM based on a preliminary trial. Chicks were monitored individually for 4 h for the
presence of hypnosis from thiopental sodium through the lack of a righting reflex, and then doses of thiopental sodium were decreased or increased by 5 mg/kg depending on the presence or absence of hypnosis.

Dose-response of thiopental sodium hypnosis in the chick model

Thiopental sodium hypnosis (lack of a righting reaction) were monitored in three groups of chicks (six chicks/group) injected with thiopental sodium at doses of 18, 36, or 72 mg/kg, IM. These doses were acquired from the ED50 values of thiopental sodium from an earlier experiment in chicks (that resemble ED25, ED50, and ED100 of thiopental sodium). Chicks were monitored individually to record the beginning of hypnosis, its duration, and the recovery time from hypnosis with a return to normal movement in all groups (Roder et al., 1993; Al-Zubaidy and Mohammad, 2005).

Hypnotic and analgesic efficacy and body indices of thiopental sodium: effect of meloxicam coadministration in the chick model

A. Effect of meloxicam coadministration on thiopental sodium analgesia

The first group received only a thiopental sodium injection (72 mg/kg, IM) whereas the second group received a combination of thiopental sodium and meloxicam (72 and 1 mg/kg, IM). The dosage of thiopental sodium was selected based on the results of the prior two experiments (dose-response and ED50 of thiopental sodium) while the dose of meloxicam was selected from another study in chickens (Souza et al., 2017). The voltage current of the electro-stimulator device produces an ache feeling (distress call) that was documented prior to injection and after 15 min of treatment for each chick model. Other documented data were analgesic percentage and delta voltage for all experimental groups.

B. Effect of meloxicam coadministration on thiopental sodium hypnosis

Two groups of chicks (6 chicks/group) were included. The first group receive only a thiopental sodium injection at a dose of 72 mg/kg, IM, while the second group received a combined injection of thiopental sodium and meloxicam at a dose of 72 and 1 mg/kg, IM. The beginning, duration, and recovery from hypnosis to normal motion were documented in both groups for each chick model.

C. Effect of thiopental sodium alone or in combination with meloxicam on respiratory rate and body temperature

Before and thirty minutes after the thiopental sodium injection (72 mg/kg, IM) or the combined injection of thiopental sodium and meloxicam (at 72 and 1 mg/kg, IM), respiratory rate (per minute) was measured at dorsal recumbency for every chick. Body temperature was measured from the cloaca using a digital thermometer for both treated groups (6 chicks per group).

Measurement of serum GPT and GOT concentration in the chick model treated with thiopental sodium or a combination of thiopental sodium and meloxicam

After 4 hours of thiopental sodium injection (72 mg/kg, IM) or thiopental sodium plus meloxicam (72 and 1 mg/kg, IM), blood samples were acquired from the jugular vein from both groups (6 chicks/group). Blood samples were centrifuged (3000 rpm for 15 minutes) in gel tubes to obtain serum, and serum was chilled pending analysis during 24 hours. Serum GPT (Reitman and Frankel, 1957) and GOT (Plummer, 1987) concentrations...
were determined (in units/L) with the specified kit (Biolabs, France) using the chemistry analyser device (Genotek, USA).

Statistics
Parametric statistical analysis included one-way statistical analysis of minimum significant dissimilarity, whereas paired and unpaired student T-test was used to compare the means of the two groups (Katz, 2006; Petrie and Watson, 2013). Non-parametric data (hypnosis and analgesic percentages) were examined by the Fisher test, and Mann-Whitney test used to analyse the delta voltages (Katz, 2006). The significance level was set at \( P<0.05 \).

### Results

#### Dose-response of hypnosis produced by thiopental sodium in the chick model

In summary, thiopental sodium injections at doses of 18, 36 or 72 mg/kg, IM yielded narcosis in a dose-dependent manner. The beginning of hypnosis (lack of the righting reflex) was rapid through 3-17 min. while the duration of hypnosis was between 21-157 min. Recovery from hypnosis of thiopental sodium persisted for 53-192 min., depending on the dose of thiopental sodium used in this experiment (Table 2).

#### Effect of meloxicam on the anaesthetic potency of thiopental sodium and body indices in the chick model

The analgesic effect after injection of the thiopental sodium and meloxicam mixture was measured for 15 minutes and showed that there was a significant

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Table 1-A. Analgesic ED\(_{50}\) value of thiopental sodium in the chick model

| Parameter     | Results       |
|---------------|---------------|
| Analgesic ED\(_{50}\) | 35.85 mg/kg, IM |
| Dosage range  | 30-40 mg/kg, IM |
| First dosage  | 40 mg/kg       |
| Last dosage   | 35 mg/kg       |
| ± in the dosages | 5 mg/kg       |
| Overall chick used | 6 (XXOXO)\(^*\) |

\(^*\)X= analgesia; O= no analgesia

Table 1-B. Hypnotic ED\(_{50}\) of thiopental sodium in the chick model

| Parameter     | Results       |
|---------------|---------------|
| Hypnotic ED\(_{50}\) | 34.40 mg/kg, IM |
| Dosage range  | 30-40 mg      |
| First dosage  | 40 mg/kg      |
| Last dosage   | 30 mg/kg      |
| ± in the dosages | 5 mg/kg      |
| Overall chick used | 5 (XOXOX)\(^*\) |

\(^*\)X= hypnosis; O= no hypnosis
increase in the antinociceptive efficacy in comparison to the group treated solely with thiopental sodium. Meanwhile, there was a significant increase in analgesic efficiency and a significant increase in delta voltage current creating pain in comparison with the group receiving only thiopental sodium (Table 3-A).

### Table 2. The dose-response related to hypnosis for various thiopental sodium dosages in the chick model

| Thiopental sodium (mg/kg, IM) | Hypnosis |  |  |  |
|-----------------------------|----------|---|---|---|
|                            | Beginning (min.) | Duration (min.) | Recovery (min.) |
| 18                         | 17.60 ± 3.92 | 21.80 ± 1.83 | 53.40 ± 7.91 |
| 36                         | 5.83 ± 0.87 * | 81.33 ± 2.81 * | 121.83 ± 11.11 * |
| 72                         | 3.00 ± 0.52 * | 157.17 ± 6.77 *<sup>a</sup> | 192.50 ± 4.87 *<sup>a</sup> |

Data expressed as Mean ± SE for six chicks per dose group
* significantly different from thiopental sodium (18 mg/kg, IM); P<0.05
<sup>a</sup> significantly different from thiopental sodium (36 mg/kg, IM); P<0.05

### Table 3-A. Analgesia produced by thiopental sodium with or without meloxicam in the chick model

| Treated groups | Analgesia % | Voltage (volt) before injection | Voltage (volt) after injection | Delta Voltage |
|---------------|-------------|---------------------------------|---------------------------------|---------------|
| Thiopental sodium (positive control) | 100 | 7.00 ± 0.45 | 16.50 ± 0.76 * | 9.50 ± 0.56 |
| Thiopental sodium and meloxicam | 100 | 7.00 ± 0.26 | 24.50 ± 0.22 *<sup>a</sup> | 17.50 ± 0.43 *<sup>a</sup> |

Data expressed as Mean ± SE for six chicks per group
Pain elicited via electro-stimulator was documented prior to and 15 minutes after thiopental sodium treatment (72 mg/kg, IM) with or without meloxicam (1 mg/kg, IM)
* significantly different from thiopental sodium alone (P<0.05)
<sup>a</sup> significantly different from pre-treatment voltage in the same group (P<0.05)

### Table 3-B. Hypnosis from thiopental sodium with or without meloxicam in the chick model

| Treated groups | % Hypnosis | Hypnosis |  |  |  |
|---------------|------------|----------|---|---|---|
|               |            | Beginning (minute) | Duration (minute) | Recovery (minute) |
| Thiopental sodium (positive control) | 100 | 4.50 ± 0.34 | 77.33 ± 5.48 | 128.67 ± 14.96 |
| Thiopental sodium and meloxicam | 100 | 1.17 ± 0.17<sup>+</sup> | 153.00 ± 3.35<sup>+</sup> | 197.83 ± 3.70<sup>+</sup> |

Data expressed as Mean ± SE for six chicks per group
Thiopental sodium administered parenterally (72 mg/kg, IM) with or without meloxicam (1 mg/kg, IM)
* significantly different from thiopental sodium alone group (P<0.05)

### B. Meloxicam coadministration and the effect on hypnosis produced by thiopental sodium

The combined mixture of thiopental sodium and meloxicam (72 and 1 mg/kg, IM) significantly shortened the beginning of the hypnotic effect and raised the duration, along with a significant elevation in the recovery time from the hypnosis.
when compared with the group receiving thiopental sodium alone (Table 3-B).

**C. Effect of thiopental sodium alone or in combination with meloxicam on respiratory rate and body temperature**

The findings in Table 3-C show that the respiratory rate and body temperature in chicks under narcotics were significantly inhibited in both groups receiving thiopental sodium alone or in combination with meloxicam in comparison with values before injection. However, the combination of thiopental sodium and meloxicam proved to have a significant deleterious effect on respiration in comparison with the experimental group injected only with thiopental sodium. Narcosis with thiopental sodium is characterized by a deep sleep with persistent leg movements and paddling reflex, whereas narcotics produced by the thiopental sodium-meloxicam coadministration is shallow and results in a quiet sleep with deep breathing.

**Serum GPT and GOT concentrations for chicks treated with thiopental sodium alone or thiopental sodium plus meloxicam**

Table 4 shows that there were no significant differences in serum GPT and GOT concentrations between the group receiving thiopental sodium alone and those treated with a combination of thiopental sodium and meloxicam.

**Discussion**

There are no prior studies dealing with the anaesthetic action profile induced by thiopental sodium and the effect of meloxicam coadministration in the 10-15 day-old chick model. Therefore, this is the first experiment of this kind. The aim of the study was to use and authenticate thiopental sodium in chicks for induction of anaesthesia and to determine the possible valuable

### Table 3-C. Respiratory rate and body temperature of thiopental sodium with or without meloxicam in the chick model

| Groups                                  | Respiratory rate (per min.) | Body temperature (°C) |
|-----------------------------------------|-----------------------------|-----------------------|
|                                         | Before 30 min. | After 30 min. | Before 30 min. | After 30 min. |
| Thiopental sodium alone (positive control) | 82.50 ± 0.92 | 70.17 ± 2.23 | 40.53 ± 0.02 | 37.35 ± 0.13 * |
| Thiopental sodium and meloxicam         | 82.17 ± 1.72 | 57.83 ± 1.08 * | 40.47 ± 0.02 | 37.28 ± 0.06 * |

Data expressed as Mean ± SE for six chicks per group
Thiopental sodium was given (72 mg/kg, IM) with or without meloxicam (1 mg/kg, IM)
* significant difference from thiopental sodium alone group at $P<0.05$
* significant difference from the pre-treatment for the same group ($P<0.05$)

### Table 4. Serum GPT and GOT concentrations for chicks treated with thiopental sodium alone or thiopental sodium plus meloxicam

| Treated groups                             | GPT [U/L] | GOT [U/L] |
|--------------------------------------------|-----------|-----------|
| Thiopental sodium (positive control)       | 12.33 ± 0.95 | 222.67 ± 2.06 |
| Thiopental sodium and meloxicam            | 13.50 ± 0.89 | 220.50 ± 2.58 |

Data expressed as Mean ± SE for six chicks per group
Thiopental sodium administered (72 mg/kg, IM) with or without meloxicam (1 mg/kg, IM)
Effect of meloxicam coadministration on the anaesthetic potency of thiopental sodium in a chick model

Učinak zajedničke primjene meloksikama na anestetsko djelovanje natrijevog tiopentala na modelu pilića

Effect of meloxicam on the anaesthetic characterization of thiopental sodium for use in the veterinary field. Thiopental sodium is considered a general anaesthetic that stimulates rapid induction of anaesthesia by increasing the GABA neurotransmitter effect on the GABA$_A$ receptor subtype, causing CNS depression (Finkel et al., 2009; Flecknell, 2009; White and Trevor, 2009). Thiopental sodium has a good hypnotic effect with occasionally less analgesic efficiency (Finkel et al., 2009; White and Trevor, 2009), whereas thiopental sodium is a safe medication of choice for producing anaesthesia since it possesses many benefits, including a familiar mechanism of action, protection from myocardial and cerebral ischemia, decreasing histamine release with a uniquely stable hemodynamic status during anaesthesia (Butera et al., 1980; Atasoy et al., 1993) and was found to induce more efficient anaesthesia than other barbiturates (Shaaban et al., 2018). Furthermore, meloxicam may be preferred for use in the veterinary field to induce balanced anaesthesia categorized by good hypnotic and analgesic effects because its analgesic, antipyretic, and anti-inflammatory effects are the result of COX inhibition (Smyth and FitzGerald, 2009; Hilal-Dandan and Brunton, 2014). The results presented here showed the anaesthetic profile of thiopental sodium in the chick model by determining the analgesic and hypnotic ED$_{50}$ values, and by determining the hypnotic dose-response for thiopental sodium. The study results show that thiopental sodium in combination with meloxicam produce balanced, reliable, and efficient anaesthesia. The thiopental sodium-meloxicam combination maximizes the hypnotic effect and increased analgesic efficacy, and a decrease in the doses of both drugs minimized the side effects when compared with thiopental sodium alone. This is of beneficial importance for the use of this combination in veterinary medicine (Mohammed et al., 2011; Brohi et al., 2019). The likely pharmacokinetic interaction between thiopental sodium and meloxicam may be regarded to the ability of meloxicam to strongly bind with plasma proteins (about 99%) (Turck et al., 1996), which may displace thiopental sodium from its binding sites on plasma proteins and make it more available to act centrally on GABA receptors. The findings of this study revealed that respiratory rate and body temperature in narcotic chicks were significantly inhibited in both groups receiving thiopental sodium alone or in coadministration with meloxicam in comparison with their values prior to injection, while the combination of thiopental sodium and meloxicam had a significant and deleterious effect on the respiratory centre in comparison to the group receiving only thiopental sodium. This is because of the potentiated inhibition caused by the pharmacokinetic interaction between thiopental sodium and meloxicam, which increases its concentration and activity on GABA receptors and exaggerates its deleterious activity on the respiratory and vasomotor centres in the brain, as confirmed in several species (Hikasa et al., 1993; Sumitraa et al., 2004; Dalir-Naghadeh et al., 2006; Abd-Almaseeh, 2008; Ferreira et al., 2013; Ninu et al., 2015; Biswas et al., 2017; Brohi et al., 2019). Serum GPT and GOT concentrations were near their normal values of chicks, as reported elsewhere (Cruz et al., 2018) and there is evidently no significant liver or tissue damages between either thiopental sodium and thiopental sodium plus meloxicam groups in relation to serum GPT and GOT concentrations. This is another reason for using this combination for inducing prolonged and enhanced anaesthesia in the chicks.
Conclusions

The outcomes of this study support the prospect of using thiopental sodium as an anaesthetic agent for veterinary surgical procedures in chickens that may be enhanced when used in combination with meloxicam to produce worthy, consistent, and proficient anaesthesia.

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Učinak zajedničke primjene meloksikama na anestetsko djelovanje natrijevog tiopentala na modelu pilića

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Malo se studija bavi anestetskim djelovanjem induciranim natrijevim tiopentalom i učinkom kombiniranja s meloksikamom (visoki stupanj vezivanja na proteine u plazmi) u pilića starih 10-15 dana. Srednje učinkovite analgete doza (ED50) ustvrđena je kao 35,85 mg/kg intramuskuarno gore-dolje rutinom, dok je hipnotička ED50 vrijednost bila 34,40 mg/kg, intramuskuarno na modelu pilića. Injekcija natrijevog pentanola (18, 36 i 72 mg/kg, intramuskuarno) stvara značajni hipnotički učinak ovisno o dozi u pilića određivanjem početka izostanka refleksa uspravljanja i tranja u vremenu oporavka. Kombinacija natrijevog tiopentanola i meloksikama (72 i 1 mg/kg, intramuskuarno), skratila je početak hipnoze, značajno produljila njezino trajanje uz značajno produljenje vremena oporavka od hipnotičkog učinka u usporedbi sa skupinom koja je primala samo natrijev tiopentanol. U isto vrijeme ista je kombinacija izazvala značajan porast analgetskog postotka i učinkovitosti uz značajan porast napona stvaranja električne simulacije za induciranje osjeta bolja. Uočeno je da poremećaj koncentracija glutamat-piruvat transaminaza (GPT) i glutamat-oksaloacetat transaminaze (GOT) u krvi s tjelesnom temperaturom, osim značajne promjene respiratorne frekvencije između dvije navedene skupine. Rezultati ove studije govore u prilog uporabe natrijevog tiopentanola kao anestetskog sredstva za veterinarske kirurške postupke u pilića čiji se učinci mogu pojačati uporabom meloksikama u svrhu postizanja dobre, dosljedne i učinkovite anestezije.

Ključne riječi: analgезija, model pilića, hipnoza, meloksikan, natrijej tiopentanal