Administration of ketamine with the central and peripheral analgesics for induction of balanced anesthesia in the chicks

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Abstract. The aim of this study was to induce balanced anesthesia characterized by good hypnosis, analgesia and reducing the side effects of the drugs at the same time, so that, the effect of ketamine administration with both morphine and aspirin was studied in 90 chicks at 10-21 day-old and compare it with the positive control group that injected with ketamine alone. Ketamine injection at 7.5, 15 and 30 mg/kg, i.m. induce loss of righting reflex (anesthesia or hypnosis) in a dose dependent manner. The onset of anesthesia is decreased and its duration and recovery from anesthesia increased significantly in the groups that administered ketamine and morphine or ketamine and aspirin or ketamine, morphine and aspirin combination when compared to ketamine alone (positive control group). The less onset of anesthesia with enhanced duration and recovery from it were significantly when ketamine injected at 15 mg/kg, i.m. mixed with morphine and aspirin at 5 and 100 mg/kg, i.m., respectively in the chicks. This combination also caused a significantly increased in the analgesic effect through its significant elevation of the voltage after injection when compared with its value before injection as well as the significant increase in the delta voltage. The result of this study recommended the induction of balanced anesthesia, which characterized by good hypnosis and analgesic efficacy through administering ketamine with central (morphine) and peripheral (aspirin) analgesics.

1. Introduction

Ketamine is a well-known drug used widely in both human and veterinary medicine to induce anesthesia for surgical purposes by inhibiting the central nervous system, leading to sleep and pain loss since ketamine produces its hypnotic and analgesic effects through its antagonistic effect on the N-methyl D-aspartate receptor in the central nervous system, causing a decrease in the amount of calcium entering the neurons that leads to inhibition of the central nervous system [1; 2]. Morphine is opioid (Mu) receptor agonist and it is used in both large and small animals due to its potency, effectiveness and pain-relieving effects through its inhibition of the central and peripheral nervous system and this inhibition occurs by stimulating opioid receptors in the nerve terminals and sensory nerve plexuses [3; 4]. On the other hand, aspirin belongs to a non-steroidal anti-inflammatory drugs (NSAIDs), which inhibits the cyclooxygenase enzyme, that is important in the production of prostaglandin from arachidonic acid, leading to peripheral pain relief [5; 6]. Despite its widespread use, ketamine has poor analgesic and weak muscle relaxing effects, which affects the efficacy of
anesthesia and surgical operations. In addition, the mixture of morphine and ketamine are used to induce reliable anesthesia by producing good hypnosis and analgesia [3; 4].

The aim of this study was to investigate the effect of ketamine alone or with and without these two analgesics (morphine and aspirin) and to find out the best doses of these drugs to induce balanced anesthesia in the chicks model.

2. Materials and Methods

2.1. Animals
The broiler chicks were brought from the local hatcheries at one day old of both sexes (90 chicks) and placed in the cages with appropriate conditions for breeding at the temperature 32-35 ºC. The proper ventilation and constant lighting were applied with feed and water ad libitum. The chicks were weighing between 70-150 g and they randomly distributed to the experimental groups with 6 chicks per group and the experiments were carried out in 10-21 days old. The ethical consideration criteria are conducted through the follow-up and t scientific committee of the College of Veterinary Medicine/University of Mosul and Dohuk and has authenticated this research in addition to the use of experimental chicks.

2.2. Preparation of drugs and injection method
The doses required of ketamine (5%, Hameln pharmaceutics, Germany) and morphine (1%, Mayne Pharmaceuticals, Australia) were prepared by using physiological normal saline solution and aspirin (Sanofi Winthrop Industrie, France) were diluted by distilled water. The amount of saline and distilled water added will depend on the concentration (dose) of the drug required. The injection volume for each of these drugs, saline solution and distilled water were 5 ml/kg body weight for chicks and has been injected into the pectoral muscle in the chest area [7; 8].

2.3. Determination of the response to the various doses of ketamine in chickens
The three doses of ketamine (7.5, 15 and 30 mg/kg) were chosen according to a previous study at the chicks [7; 8]. This trial is intended as a first step to determine the best dose of ketamine for induction of anesthesia to be used in subsequent trials with morphine and aspirin. The onset of anesthesia (Hypnosis) was recorded which is the period from injection of ketamine until the loss of righting reflex in each of the chicks alone. The duration of anesthesia (hypnosis) was calculated from the loss of the righting reflex until the chicks returned and corrected their body to normal, whereas the recovery period were recorded which is the period between the start of anesthesia and the return of chicks to movement and normal activity [9; 10; 11].

2.4. Measuring the anesthetic effect (hypnosis) of ketamine alone or with morphine or aspirin in the chicks
Twenty-four of the 10 to 21-days old chicks were used with six chicks per group and divided into the following groups:
1. Ketamine alone (control): Chicks were injected with ketamine alone at a dose of 15 mg/kg, i.m. and injection of 5 ml/kg, for both saline and distilled water.
2. Ketamine and morphine group: Chicks were injected with ketamine with a dose of 15 mg/kg with morphine at 5 mg/kg, i.m. and injection of 5 ml/kg, i.m. of distilled water.
3. Ketamine and aspirin group: ketamine was used at 15 mg/kg with aspirin at a dose of 100 mg/kg, i.m. and 5 ml/kg, i.m. of saline solution.
4. Ketamine, morphine and aspirin group: injection of ketamine, morphine and aspirin at doses of 15, 5 and 100 mg/kg i.m., respectively.
5. The dose of ketamine was chosen based on the previous experiment while the dose of morphine was selected based on other study in chickens [12] and preliminary investigation and aspirin [13].
The onset, duration of anesthesia and its recovery was recorded for each chick individually as in the previous experiment.

2.5. Evaluating the analgesic effect of ketamine alone or with morphine or aspirin in the chicks
The other same old and numbers of chicks in the previous experiments were used with 6 chicks per group. Electrostimulation was applied via electro-stimulator (Scientific and Research Ltd, England) for causing pain sensation characterized by a distress call in the chicks. The device could be used to evaluate the pain-dwelling function of chicks [7; 8]. The stimulation of the electro-stimulator was set based on initial experiments in the chicks at the frequency of 50 Hertz and the width of the electrical impulse of 5 MilliAmpere/Second with repetitive pulses.

The experimental groups were divided as the previous experiment and the pain-induced voltages were measured before and after 5 minutes of i.m. injection of ketamine alone (15 mg/kg) or with morphine (5 mg/kg) or aspirin (100 mg/kg). The device was applied to the skin via two probes in the area under the wing of the chicks with moisturizing the area with water to ensure better delivery of electricity and then increased voltages until the occurrence of distress call as an indicator of pain in the chicks.

The result recorded as no analgesia, i.e., the same or decrease in the voltages recorded after injection in comparison to the voltages measured before the injection. Analgesia will be indicated when there was an increase in the voltage after injection related to the voltage measured before the injection. The higher the voltages after injection than the pre-injection voltages, the higher occurrence of analgesia in that group.

2.6. The effect of ketamine alone or with morphine or aspirin at the glucose level, aspartate transaminase (AST), and Alanine Transaminase (ALT) in plasma of the chicks
Blood samples were collected from the jugular vein of the chicks (six chicks/group) after 4 hours of injecting the above drugs with the same doses in the previous experiment. The concentration of glucose in plasma was measured using an analytical kit (Syrbio Paris, France) by measuring the absorption at spectrophotometer at a wavelength of 505 nanometers [14]. The concentrations of both AST and ALT were also measured in plasma using kits (BioMerieux, France) and the absorption at spectrophotometer device at a wavelength of 505 nanometers [15; 16].

2.7. Statistical analysis
The data were statistically analyzed for more than three groups using the One Way Analysis of Variance test. The results were subjected to the Least Significant Difference Test [17; 18]. The two groups were analyzed using the Paired student T-test. Non-parametric data were statistically analyzed using the Mann-Whitney-U-test Package [18; 19]. The mean difference of all tests at a significant level was less than 0.05.

3. Results and Discussions

3.1. Response to various doses of ketamine in the chicks
Injection of ketamine resulted in anesthesia (hypnosis) of 100% of the chicks and was detected by the loss of the reflex. The onset of anesthesia was significantly occurred in all groups that injected with a different dose of ketamine (7.5, 15 and 30 mg/kg, i.m.) between 1.00 and 9.33 minutes in response to the dose. The duration of anesthesia was dose-dependent between 7.83 to 28.17 minutes, whereas there is a significantly increased in the recovery period between 8.67 and 37.00 minutes after starting of anesthesia and depend on the dose of ketamine Table 1.

Table 1 also shows that the lowest and best dose of ketamine, which acts as an anesthetic, is a dose of 15 mg/kg i.m. Therefore, it was used in subsequent experiments with both morphine and aspirin to induce balanced anesthesia.

Ketamine is an anesthetic drug that inhibits the central nervous system and works to induce hypnosis and pain loss which facilitates the surgical operations by its action of blocking the N-
methyl-D-aspartate (N-MDA) receptor in the central nervous system causing a decrease in the amount of calcium entering the neurons [1; 2].

**Table 1. Response to various doses of ketamine in the chicks**

| Ketamine (mg/kg, i.m.) | Onset (min.) | Duration (min.) | Recovery (min.) |
|------------------------|--------------|-----------------|-----------------|
| 7.5                    | 9.33 ± 2.29  | 7.83 ± 0.70     | 8.67 ± 1.38     |
| 15                     | 1.33 ± 0.21* | 15.00 ± 1.37*   | 23.50 ± 3.47*   |
| 30                     | 1.00 ± 0.00* | 28.17 ± 3.63*   | 37.00 ± 2.05*   |

Values are the mean ± the standard error of six chicks/group.

* The value was significantly different from the dose of ketamine 7.5 mg/kg, i.m. at a significant level (p <0.05).
* The value was significantly different compared to the dose of ketamine 15 mg/kg, i.m. at a significant level (p <0.05).

3.2. *The anesthetic effect of ketamine alone or with morphine or aspirin in chicks*

The injection of ketamine alone (control) or in combination with both morphine and aspirin causes the occurrence of loss of reflex (anesthesia) in 100% of the chicks.

Anesthesia began with all groups of the chicks approximately between 1-2 minutes and the duration of anesthesia between 16.33 - 189.67 minutes while the recovery from it between 26.17 to 213.67 minutes after the starting of anesthesia (Table 2).

It is noted from Table 2 that there are differences between ketamine-injected groups alone and those injected with ketamine with both morphine or aspirin for the startup, duration and recovery from anesthesia.

The onset of anesthesia is decreased and its duration and recovery from it were increased significantly in groups that injected with ketamine and morphine or ketamine and aspirin or ketamine with both morphine and aspirin when compared to the ketamine alone (positive control group).

It is also noted that the least time to start anesthesia with increased its duration and recovery from it was significant when injecting ketamine with a mixture of compound (morphine and aspirin), so this group will be selected to examine the analgesic effect and to be compared with the previous groups.

Due to the widespread use of ketamine in large and small animals and the lack of studies on the effect of ketamine by using the central (morphine) and peripheral (aspirin) analgesic drugs to induce balanced anesthesia (characterized by unconsciousness, good muscle relaxation, good analgesia and hyporeflexia) in the chicks, so that, the aim of this study was to demonstrate these effects of ketamine alone or with these analgesics and to find out the best doses of these drugs in chicks model which cause the best anesthesia to be applied to surgical operations. Morphine is frequently used with ketamine to induce enhanced anesthesia, which characterized by inducing unconsciousness and good analgesia because ketamine does not induce good analgesia and muscle relaxation alone [3; 4] as reported in previous studies in rats [20], cattle [21], horses [22], donkeys [23], goats [24] and birds [25]. The use of morphine in combination with ketamine due to its stimulation of the opioid (Mu) receptor that causing analgesia and leading to inhibition of the neuronal activity in the central nervous system and causing further additive inhibition to the nervous system [3; 4; 12; 26].
Table 2. The anesthetic (hypnotic) effect of ketamine alone or with morphine or aspirin in the chicks

| Groups                      | Anesthesia (Hypnosis) |          |          |
|-----------------------------|------------------------|----------|----------|
|                             | Onset (min.)           | Duration (min.) | Recovery (min.) |
| Ketamine alone (positive control) | 2.00±0.45             | 16.33±1.82 | 26.17±2.47 |
| Ketamine+Morphine           | 1.00±0.00              | 118.83±3.08* | 166.50±5.42* |
| Ketamine+Aspirin            | 1.83±0.54              | 36.00±6.32*  | 95.33±4.88*  |
| Ketamine+Morphine+Aspirin   | 1.00±0.00              | 189.67±7.64**  | 213.83±3.45** |

Values are the mean ± the standard error of six chicks per group.

Ketamine, morphine and aspirin were injected at doses 15, 5, 100 mg/kg, i.m., respectively.

Morphine, aspirin, saline solution and distilled water were injected immediately after injection of ketamine.

* The value was significantly different compared to ketamine alone (control) at a significant level (p <0.05).
+ The value was significantly different compared to the group of ketamine and morphine at a significant level (p <0.05).
# The value was significantly different from the ketamine and aspirin group at a significant level (p <0.05).

3.3. The analgesic effect of ketamine alone or with morphine or aspirin in the chicks

The administration of ketamine with both morphine and aspirin has been associated with analgesic effect with an increase in delta voltages and a significant increase in pain-induced voltages when compared to pre-injection pain voltages Table 3.

Table 3 also shows that the groups that injected with a mixture of both morphine, aspirin and ketamine has a significantly increased in the analgesia when compared to the ketamine alone. The best analgesia obtained was when ketamine injected with both morphine and aspirin.

Previous studies have not indicated the use of aspirin with ketamine or in combination with ketamine and morphine to increase analgesia and the possibility of increasing hypnotic time and analgesia during anesthesia required for relatively longer surgical procedures and also for reducing the doses used for each of these drugs and thus reducing the side effects of these drugs so, the idea of conducting this study was built. The results of this study showed that the use of ketamine in combination with both morphine and aspirin resulted in an increase in analgesia and accompanied by an increase in hypnosis during anesthesia which is likely to be used for induction of anesthesia to perform surgical operations. Ketamine was used with a dose of 15 mg/kg, i.m. [7; 8] with morphine at 5 mg/kg [12] or aspirin at a dose of 100 mg/kg, i.m. [13] that causes the loss of righting reflex in the chicks which is one of the signs of anesthesia or hypnosis. This suggests that these drugs have worked to influence different regions of the brain responsible for balance and correcting the body and thus the incidence of sleep for a longer period with increased analgesia than if they were given separately as found in a previous study in guinea pigs [27; 28]. In addition, ketamine result in the reduction of calcium ion inside the nerve cell and this is important for neurotransmitter releases, which contributes to more inhibition of the central nervous system and enhanced the analgesia when used with aspirin with both ketamine and morphine because all of these drugs works to bring about good analgesia with different working mechanism in the body of the organism.
Table 3. The analgesic effect of ketamine alone or with morphine or aspirin in the chicks

| Groups                                      | Before injection | Pain induced (Volts) | Delta Voltage |
|---------------------------------------------|------------------|----------------------|--------------|
|                                             | Before injection | After injection      |              |
| Ketamine alone (Positive control)           | 5.83±0.48        | 7.50±0.89            | 1.67±0.84    |
| Ketamine+ Morphine                          | 6.50±0.34        | 24.50±0.22*†        | 18.00±0.34*  |
| Ketamine+ Aspirin                           | 6.00±0.45        | 14.50±0.72*†‡       | 8.50±1.00*+  |
| Ketamine+Morphine+Aspirin                   | 4.83±0.48        | 25.00±0.00*‡#‡       | 20.17±0.48*+#|

Values are the mean ± the standard error of six chicks per group.

Ketamine, morphine and aspirin were injected at doses 15, 5, 100 mg/kg, i.m. respectively.

* The value was significantly different compared to ketamine alone (control) at a significant level (p <0.05).
† The value was significantly different compared to the group of ketamine and morphine at a significant level (p <0.05).
‡ The value was significantly different from the ketamine and aspirin group at a significant level (p <0.05).
# The value was significantly different from the voltage before injection at a significant level (p <0.05).

3.4. Effect of ketamine injection alone or with morphine or aspirin at the level of glucose and AST and ALT enzymes in plasma of the chicks

Table 4 shows that the concentration of both glucose and AST and ALT in the plasma of the chicks did not differ significantly between the ketamine-treated groups alone or when given with either morphine or aspirin, although, there were slight differences in concentrations when compared between the groups of chicks.

The study showed that the concentrations of glucose and AST and ALT in the plasma of the chicks did not differ significantly between the groups treated with ketamine alone or when given with both morphine or aspirin, although there were slight differences in concentrations when comparing the different groups and this indicates that the combination of ketamine mixed with either aspirin or morphine or both has slight liver and metabolic damage when compared to using of ketamine alone in the chicks.

Table 4. Effect of ketamine alone or with morphine or aspirin at the glucose level and AST and ALT enzymes in plasma of the chicks

| Groups                                      | ALT (IU/L)   | AST (IU/L)   | Plasma Glucose (mg/dl) |
|---------------------------------------------|--------------|--------------|------------------------|
| Ketamine alone (Positive control)           | 22.17±3.07   | 56.17±4.47   | 123.67±13.97           |
| Ketamine+Morphine                           | 26.50±3.42   | 67.50±7.42   | 132.50±15.24           |
| Ketamine+Aspirin                            | 30.33±4.88   | 62.33±6.88   | 120.33±9.88            |
| Ketamine+Morphine+Aspirin                   | 33.83±5.45   | 71.83±7.45   | 140.83±11.45           |

Values are the mean ± the standard error of six chicks per group.

Ketamine, morphine and aspirin were injected at doses 15, 5, 100 mg/kg, i.m. respectively.

Morphine, aspirin, saline solution and distilled water were injected immediately after injection of ketamine.
4. Conclusions

The results of this study suggest the induction of balanced anesthesia, which is characterized by good anesthesia (hypnosis) and good analgesic efficacy by administering ketamine with the central (morphine) and peripheral (aspirin) analgesics to induce balanced anesthesia in the chicken model.

5. Acknowledgements

The researchers extend their thanks and appreciation to the College of Veterinary Medicine / University of Mosul and Duhok for their support by providing the adequate equipment to complete this project.

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