Efficacy of Ketamine in Combination with Four Common Analgesics on Some Hematological Factors of Broiler Chickens

Moosa Javdani1,2, Zahra Nikousefat1, Ali Ghashghaei1, Mohammad Hashemnia3*
Department of 1Clinical Sciences and 2Pathobiology, Veterinary Medicine Faculty, Razi University, 2Avian Surgery and Anesthesia Research Center, Razi University, Kermanshah, Iran
Corresponding author - m.hashemnia@razi.ac.ir

Rec. Date: Feb 04, 2014 07:19
Accept Date: Mar 24, 2014 10:07

Abstract
In order to provide information on hematological and biochemical changes in broiler chickens during anesthesia after injecting of ketamine (35 mg/kg) combined with (A) tramadol (0.2 mg/kg), (B) midazolam (0.5 mg/kg), (C) hyoscine (0.04 mg/kg), and (D) atropine (0.05 mg/kg), forty numbers of broiler chickens were allocated to four equal groups. Each group received one of ketamine combinations in the pectoral muscles. Afterwards, values of packed cell volume (PCV), WBC, HLI (Heterophil/Lymphocyte Index), aspartate aminotransferase (AST), CoE, and glucose were determined at 0, 30, and 60 minutes post injection. Results revealed that injections of different combined drugs did not cause any specific effect on PCV values except in group D that showed significant increasing of PCV in time of 30 and 60. Clear leukocytosis was recorded at 60 min post injection groups A and C. Furthermore, spending time made an increase on heterophil populations compared to lymphocytes (P≤0.05). AST levels tended to rise in groups A, B and D during the experiment and a significant increase in AST activity was recorded in group B and D at 30 and 60 min post injection. The mean glucose and CoE concentrations decreased after injections in all groups; however, the concentration of CoE was increased at 60 min post injection in group B. In conclusion, using of ketamine in anesthesia can make changes effectively in several blood parameters of broiler chickens in combination with other drugs.

Key words: Chicken, Anesthesia, Hematological Parameters

Introduction
Recognizing pain, treating it appropriately, and evaluating the efficacy of administered treatments can be one of the greatest challenges facing avian practitioners. Although there are lists of analgesics and dosages available in the literature and their combinations are commonly used in veterinary medicine to facilitate handling of small animals, there are only a few investigations that document the analgesic efficacy of these drugs in avian practice (Hall et al., 2001).

Sedation and anesthesia in avian are used based on immobilization and analgesia to reduce the nervous feeling and consciousness level (Machin, 2005). For this reason, ketamine injection is widely utilized in combination with other drugs, in order to reduce adverse effects.

Ketamine was developed in the 1960s as a safer and more predictable anesthetic than its precursor phencyclidine. It provides excellent amnesia and analgesia by blocking communication between the thalamic and limbic regions of the brain (Green and Krauss, 2000). Ketamine preserves muscle tone
maintaining protective airway reflexes and spontaneous respiration (Krauss and Green SM, 2006). Despite its obvious advantages over other agents, some practitioners are hesitant to use ketamine alone secondary to its propensity to cause vivid and frightening emergent reactions an also sympathomimetic effects and vomiting when administered in sedating doses (Green et al., 1998; Roback et al., 2005). The combination of ketamine with other anesthetics permits decrease of the dose of ketamine and can therefore allow faster recovery times.

Midazolam is a short-acting, water-soluble drug in the benzodiazepine class that is widely used a premedication for sedation; less commonly it is used for induction and maintenance of anesthesia. Midazolam has the potential to cause cardiac/respiratory depression. The most common undesirable effects are loss of respiratory volume and or fall in respiratory rate or apnoea (Kupietzky and Houpt, 1993).

It is appeared that the birds could feel pain. Pain perception in birds is mediated by neural pathways and neurotransmitters similar to mammals (Gentle and Hunter, 1991; Tanelian and Brunson, 1994). Utilization of opioids in mammalian and some avian species can reduce anesthetic doses via their analgesic effects (Curro, 1993; Curro et al., 1994; Reim and Middleton, 1995; Concannon et al., 1996). Tramadol as a synthetic, centrally acting effective opioid analgesic is used for treatment of moderate to severe acute or chronic pain (Lee et al., 1993). Pain relief efficacy of tramadol is provided by opioid (mu), serotonin, and nor-epinephrine pathways, with minimal unpleasant effects. The analgesic power of this opioid is about 10% of morphine patency following parenteral administration. Postoperative analgesic effects of tramadol are comparable with the pain relief effects of pethidine (Grond and Sablotzki, 2004). Available scientific reports about administration of tramadol in avian species are restricted.

Anticholinergic drugs are commonly given as preanaesthetic medication to reduce secretions and bradycardia. Hyoscine is an anticholinergic with central and peripheral action (Watney et al., 1987). Hyoscine can reduce airway secretions, nausea and vomiting during anesthesia with laryngeal mask airway (Alijanpor et al., 2010). Hyoscine prevented detomidine induced bradycardia and may be a useful drug combination against the bradycardia induced by this alpha-2 agonist, in horses (Valadao et al., 2000). Hyoscine broadly used as a spasmolytic in the treatment of abdominal pain in horses that has shorter-acting muscarinic cholinergic blocking effects compared to atropine and is effective in relaxing the bowel wall to prevent contraction (Davies and Gerring, 1983; Adams et al., 1984). Some researchers believed that hyoscine can prevent the bradyarrhythmia produced by romifidine in horses and also intravenous administration of hyoscine can increase arterial blood pressure and heart rate in horses anaesthetized with halothane (Borer and Clarke, 2006; Marques et al., 1998).

Atropine reduces vagally induced bradycardia and oral secretions that may block endotracheal tubes (Heard, 1997). It however may has adverse effects, the main ones being an unacceptably tachycardia,
increasing myocardial oxygen demand and making oral or respiratory secretions so tough that they make endotracheal tube obstruction even more likely. Administration of anticholinergics before or during of ocular surgery can prevent cardiac arrhythmia due to stimulation of cardio-ocular reflex (Pipo et al., 1996). The Bird heart rate will enhance following administration of atropine (Gunkel and Lafortune, 2005).

Although when analgesic drugs are administered together, synergism seems to occur; sedation and analgesia being greater than that achieved with either drug given alone (Hall et al., 2001), no information is available about their effects on hematological and biochemical parameters. Therefore, the present study evaluated several hematologic changes in broiler chickens during anesthesia after injecting of ketamine combined with tramadol, midazolam, hyoscine, and atropine.

Materials and Methods

Experimental animals and design
Forty numbers of broiler chickens were used in the study. Mean ± SD weight of the broilers was 488.58±60.02 gram. Health status was assessed by means of physical examination, a complete blood count and serum biochemical analyses; all findings were within reference ranges. This experiment was performed under the approval of the state committee on animal ethics, Razi University, Kermanshah, Iran. Also, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the protection of animals used for experimental purposes, were considered. Before experiment, food but not water was withheld for 12 hours and the broilers were randomly divided into four groups. Ten broilers were used in each group.

Group A: Injecting of ketamine (35 mg/kg) combined with tramadol (0.2 mg/kg) in the pectoral muscles.
Group B: Injecting of ketamine (35 mg/kg) combined with midazolam (0.5 mg/kg) in the pectoral muscles.
Group C: Injecting of ketamine (35 mg/kg) combined with hyoscine (0.04 mg/kg) in the pectoral muscles.
Group D: Injecting of ketamine (35 mg/kg) combined with atropine (0.05 mg/kg) in the pectoral muscles.

Afterwards, values of PCV, WBC, HLI (Heterophil/Lymphocyte Index), AST, CoE, and glucose were determined at 0, 30, and 60 minutes post injection.

Hematological and biochemical analyses
Blood samples were taken from ulnar vein and collected in vacutainers containing EDTA for hematological factors and into vacutainers without anticoagulant for biochemical analysis. The collected sera were separated by centrifugation at 750 × g for 15 min and stored at −20 C until be analyzed. Total leukocyte counts were estimated manual using a hemacytometer and Natt and Herrick’s solution. For
differential leukocyte counts, the blood smears were prepared and stained with Giemsa stain (Jain 1986). H:L (Heterophil/Lymphocyte ratio) was calculated using absolute count of heterophils divided by lymphocytes. PCV was determined using a microhaematocrit centrifuge (Labtron Co. Tehran, Iran) at 12000 × g for 5 minutes. Cortisol was analyzed with a commercial enzyme-linked immunosorbent assay (ELISA) kit (IBL, Hamburg, Germany) and an ELISA reader (Statfax, USA). Serum aspartate aminotransferase (AST) and glucose activities were measured using the Hitachi 911 chemistry analyzer (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacture’s recommendation.

Statistical analysis

Student's t-test was employed to determine statistical significance of differences between groups and ANOVA test was employed to detect a significant difference between groups means. Data were analyzed by SPSS software, version 16 and P<0.05 was accepted as statistically significant.

Results

The mean ± SD of the hematological and biochemical values from different samples of each group at 0, 30 and 60 min intervals are presented in Tables 1.

Results revealed that injections of different combined drugs did not cause any specific effect on PCV values except in group D that significant increasing of PCV was recorded at 30 and 60 min post injection. An increase in the number of WBCs is observed in the peripheral blood of broilers in group B at 30 min post injection comparing to other groups. Clear leukocytosis was recorded at 60 min post injection with more than 9.8×10^3 and 8.68×10^3/l WBCs in group A and C respectively. The changes in the number of WBC were not prominent in group D during the experiment. Heterophil/Lymphocyte index (HLI) in the WBC differential count estimated at maximum level after injection of midazolam and atropine in time 60 (0.93±0.02 and 0.91±0.05 respectively). AST levels tended to rise in groups A, B and D during the experiment and a significant increase in AST activity was recorded in group B and D at 30 and 60 min post injection. However, the AST levels showed a sharp increase at 30 min post injection in group C and decreased thereafter. The mean glucose and CoE concentrations decreased after injections in all groups; however, the concentration of CoE was increased at 60 min post injection in group B.

Discussion

Pain perception in birds is believed to be analogous to that of mammals. Thus, invasive and painful procedures should always be accompanied by appropriate analgesia and anesthesia (Machin, 2005). Ketamine is a dissociative anesthetic and is often combined with sedatives such as α2-agonists and benzodiazepines for premedication or general anesthesia for minor procedures. Lower doses of ketamine can be useful for preemptive analgesia in major surgeries and also for postoperative analgesia. Although ketamine prevents sharp, superficial pain effectively, it does not control visceral, dull pain, so ketamine alone is not adequate for laparotomies or orthopedic surgery.
(Lamont et al., 2000). The combination of ketamine with other anesthetics permits decrease of the dose of ketamine and can therefore allow faster recovery times.

Table 1 Mean ± SD of hematological and biochemical values in different groups.

<table>
<thead>
<tr>
<th>Ketamine</th>
<th>PCV (%)</th>
<th>WBC (/l)</th>
<th>HLI</th>
<th>AST (U/l)</th>
<th>Cortisol (mcg/dl)</th>
<th>Glu (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30</td>
<td>60</td>
<td>0</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>TRAMADOL</td>
<td>25.2±0</td>
<td>25.6±0</td>
<td>25.2</td>
<td>1.12±0</td>
<td>1.10±0</td>
<td>9.8±1</td>
</tr>
<tr>
<td></td>
<td>0.55±1</td>
<td>0.99±1</td>
<td>.3±1</td>
<td>10±5</td>
<td>10±5</td>
<td>0±28</td>
</tr>
<tr>
<td>MIDAZOLAM</td>
<td>25.2±1</td>
<td>25.4±0</td>
<td>25.8</td>
<td>1.12±0</td>
<td>9.98±0</td>
<td>8.64±0</td>
</tr>
<tr>
<td></td>
<td>.03±1</td>
<td>.53±1</td>
<td>.65±1</td>
<td>10±5</td>
<td>10±5</td>
<td>10±3</td>
</tr>
<tr>
<td>HYOSCINE</td>
<td>23.3±0</td>
<td>26.2±0</td>
<td>26.3</td>
<td>1.15±0</td>
<td>1.06±0</td>
<td>8.68±0</td>
</tr>
<tr>
<td></td>
<td>.57±1</td>
<td>.9±1</td>
<td>.25±1</td>
<td>10±5</td>
<td>10±3</td>
<td>10±4</td>
</tr>
<tr>
<td>ATROPINE</td>
<td>24.3±0</td>
<td>29.5±0</td>
<td>29.1</td>
<td>1.13±0</td>
<td>1.07±0</td>
<td>1.23±0</td>
</tr>
<tr>
<td></td>
<td>.93±1</td>
<td>.7±1</td>
<td>.78±2</td>
<td>10±5</td>
<td>10±3</td>
<td>10±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.2±0</td>
<td>.2±0</td>
<td>.2±0</td>
</tr>
</tbody>
</table>

Means within a column with different superscript letters (a, b, c) and numbers (1, 2, 3) denote significant differences between times and experimental groups, respectively. *P<0.05 was accepted as statistically significant.*

PCV packed cell volume, WBC white blood cell, HLI (Heterophil/Lymphocyte Index), AST aspartate aminotransferase, CoE cortisol Glu glucose

Combination of ketamine with xylazine (1–2.2 mg/kg) and medetomidine (60–85µg/kg) can decrease the ketamine dose to 5–10 mg/kg (Curro 1998). In fact, utilization of alpha 2 agonists can reduces the ketamine dosage significantly and so improves recovery rates, whilst enhancing levels of sedation and analgesia. The combination of ketamine with other anesthetics permits decrease of the dose of ketamine and can therefore allow faster recovery times. Diazepam (1–1.5 mg/kg) and midazolam (0.5–1.5 mg/kg) allows the ketamine dose to be decreased to 20–40 and 10 mg/kg, respectively. On the other hand, these two benzodiazepines lead to muscle relaxation and mild sedation, and reduce wing movement on recovery through a reduction of recovery time (Curro, 1998; Edling, 2005).

Midazolam is more potent and it’s recovery is quicker than diazepam and it tends not to affect mean arterial blood pressure and gases to the same extent (Edling, 2005). It has been reported that intranasal administration of midazolam can result to sedation and recumbency in Ring-necked Parakeets (Vesal and Eskandari, 2006). Midazolam can improve anesthesia quality in guinea fowl (Ajadi et al., 2009) and also...
as a premedication agent in waterfowl may exhibit periods of apnoea during mask induction of anesthesia (Ludders and Matthews, 1996).

Utilization of opioids in mammalian and some avian species can reduce anesthetic doses via their analgesic effects of them (Curro, 1993; Curro et al., 1994; Reim and Middleton, 1995; Concannon et al., 1996). Tramadol is a useful and well tolerated drug to reduce pain in patients with a risk of poor cardiopulmonary function, after surgery of the thorax or upper abdomen and also for the management of chronic pain of malignant or nonmalignant origin, particularly neuropathic pain (Grond and Sablotzki, 2004). However, available scientific reports about administration of tramadol in avian species are restricted.

In this experiment, the mean PCV value increased in group D and other combined drugs did not cause any specific effect on PCV. During the capture in mammals, the erythrocyte count, PCV, Hb concentration increased because of the effect of catecholamines on α-adrenergic receptors in the splenic capsule and the release of erythrocytes to the blood circulation. In the birds, the spleen does not provide a reservoir of erythrocytes and they have been shown to rapidly recover from the experimentally induced blood loss (Lopez-Olvera et al., 2007).

Dressen et al., (1999) reported that use of isoflurane in American kestrels (Falco sparverius) causes a mild decrease in packed cell volume, basophil concentration and plasma proteins. In another study, use of intramuscular ketamine hydrochloride in the red tailed hawks (Buteo jamaicensis) showed no significant differences in the hematocrit values of conscious birds at 10, 20 and 40 minutes after injection (Kollias and Mcleish, 1978). Infusion of ketamine-lidocaine combination in dogs did not cause a noticeable change in hemodynamic profile (Monteiro et al., 2009).

In our study, an increase in the number of WBCs is observed in groups A, B and C. The changes in the number of WBC were not prominent in group D during the experiment. A slight to moderate leukocytosis can occur in birds with excess endogenous glucocorticoids, due to the excitement caused by handling (Jain, 1993).

Although hematological response to stress varies from species to species in avian, birds with a total white blood count above the third quartile (leukocytic) had an absolute heterophilia, which may reflect more of a catecholamine-induced stress pattern rather than a corticosteroid-induced hemogram. The influence on the heterophil percentile, in addition to disease or injury has not been comprehensively described for birds. Consequently, the careful assessment of the morphology of leukocytes, particularly heterophils, is a most valuable component in the interpretation of hematology values of birds.

However, mild leukocytosis and the increase of secreted antibodies from B cell has been reported after tramadol, pethedine and atropine injection (Sacerdote et al., 2000; Salo 1977), but according to our findings, it seems that the atropine is a good choice to reduce the stress effects in birds.
Heterophil/Lymphocyte (H/L) ratio used as an index of stress in birds that measures a physiological change while cortisol cause the release of heterophils from hemopoetic system through the blood circulation and introduced as another hallmark of stress. Generally, cathecoleamines release can affect marginal pool of heterophils and cause increase of H/L index in the first changes (Gross and Siegel, 1983).

In our study, Heterophil/Lymphocyte index in WBC differential count estimated in minimum level at 30 min post injection and remained in basic range until 60 min post injection. It seems that tramadol can cause effective response on reducing stress of handling compare to others that acts via suppressing hypothalamus-cortisol. Moreover, it sustains the capacity to maintain cortisol level in basic during surgery operation. In fact, it facilates GABA effect 30 min after IM injection and return cortisol level to basic level.

AST levels tended to rise in groups A, B and D during the experiment and a significant increase in AST activity was recorded in group B and D. Turk and Casteel (1997) reported that the serum levels of ALT and AST are increased in acute hepatic degenerations. Although, AST is not liver-specific and is found in most tissues, increased serum AST activity is usually associated with liver or muscle injury.

In our study, the mean CoE and glucose concentrations decreased after injections in all groups; the lowest mean concentration of CoE was observed after injection of tramadol.

When birds exposed to a stressor, such as restrain, they become excited, cathecoleamines and consequently the corticostrone located is in the adrenal cortex is released into the blood circulation to help them increase metabolism. It is well known that glococorticoids plays an important role in the process of metabolism of animals by enhancing mobilization of carbohydrates, protein and fat from liver and muscle tissue and increase the concentration of metabolites. Therefore, the effects of glucocorticoid are very meaningful for protecting the functions of important tissues or organs when animals suffer from long term or abrupt stress (Cockrem and Silverin, 2000).

References


