Comparison the Effect of Metoclopromide, Atropine, Midazolam and Acepromazine on Capturing Stress in Pigeons (*Columba liviadomestica*)

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Abstract

In pigeons (*Columba liviadomestica*) like any other unhandled animals, release of catecholamine and corticosteroids occurs following capturing and restriction. This hormonal response can changes some hematological, biochemical and clinical parameters that known as stress features. This study determined some stress responses in means of hematologic-biochemical changes following intramuscular (IM) injection of metoclopromide (0.5 mg/kg), atropine (0.04 mg/kg), midazolam (0.2 mg/kg) and acepromazine (0.1 mg/kg) in time zero, 30 and 90 minutes after injection. For this purpose, this study was conducted on 40 healthy male pigeons. Following injection of metoclopromide or atropine, significantly higher serum cortisol level was observed. However there was a decrease in plasma cortisol concentration with midazolam. Packet cell volume was only changed increasingly in atropine group. The merely drug that kept Heterophil/Lymphocyte (H/L) index constant was midazolam whereas in other drugs group increase of this factor was observed. Higher H/L index belongs to atropine administered group after injection. Acepromazine has the ability of maintaining basic cortisol level during surgery procedure. In contrast, midazolam achieves peak of its effect about 30 minutes after intramuscular injection which can decreased cortisol to the basic level at the moment. Blood sugar increased in all groups after handling of the birds. Higher blood glucose concentration relies on internal glucocorticoids and indicates that these drugs cannot decrease glucose in the short time period. Total protein remains unchanged during short time of the study.

**Key words**: Pigeon, Metoclopromide, Atropine, Midazolam, Acepromazine

Introduction

Physical restriction is one of the key parts of keeping and caring the birds. This behaviour could be performed during transportation, physical examination and treatment of birds. Physical restriction results in anxiety and stress situation, especially in birds which were not adapted with humans. Like any other unhandled animals, release of catecholamine and corticosteroids occurs following capturing and restriction (Moberg 1987; Cockrem and Silverin 2002). This hormonal response can changes some
hematological, biochemical and clinical parameters known as stress features (Marco et al. 1997; Moe and Bakken 1997; Davis and Maerz 2008). Some studies show that stress of restriction can increase heart rate, body temperature, red blood cell count, hemoglobin concentration, hematocrit, serum glucose concentration, lactate, urea, bilirubin, sodium and chloride (Hatting 1988; Moe and Bakken 1997; Montané et al. 2003; Perego et al. 2014). In addition, some reports indicate that serum level of lactate dehydrogenase, aspartate aminotransferase and creatine kinase increased during capturing and handling (Duncan et al. 1994; Montané et al. 2003). There are some researches that introduce serum cortisol as a valuable indicator for stress (Morton et al. 1995; Waas 1999). Because of differences between animals individuality characteristic and their responses to stress, physiological changes based on animal species and type of stressful situation (López-Olvera et al. 2006).

Midazolam is a water soluble medication and a member of drug family Benzodiazepines. It becomes growingly popular in veterinary medicine due to its ease of use (Bigham Sadegh 2013). It shows characteristics of sedation and muscle relaxing with lower effects on cardiovascular system. Ajadi et al. (2009) reported that use of midazolam can improve quality of bird’s anaesthesia. Furthermore, Vesal and Eskandari (2006) introduce midazolam as an appropriate sedative for parrots. Another drug, acepromazine, is a short-act phenothiazine derivative which has central effect (post-synaptic dopamine blocker) and block alfa-adenergic receptors (Plumb 1999; Monteiro et al. 2009). Atropine is an anticholinergic alkaloid which has a prominent usage in anaesthesia. Following injection of atropine, conduction of post-ganglion cholinergic impulses ceased, especially in heart and salivary glands (Hall et al. 2001). Metoclopromide is an anti-vomiting drug that depresses central nervous system (CNS) and also has peripheral activity (Allen et al. 2002; Pang et al. 2009). Depressing CNS effect seems the only reason lead the drugs chosen for this study. The aim of this study was to assess some of the haematological and biochemical changes following handling and capturing in pigeons. In addition, effects of several drugs that frequently used in veterinary and avian medicine were also evaluated on stress status of pigeons.

One of the bird’s species that draws considerable attention for handling, capturing and physical restriction is pigeon. Hence, several haematology and biochemical changes were measured at three time intervals following intramuscular (IM) injection of metoclopromide, atropine, midazolam and acepromazine in three times. Measured parameters such as stress markers include serum cortisol, packed cell volume (PCV), total protein (TP), blood sugar (BS), heterophil, lymphocyte and heterophil/lymphocyte (H/L) index.

Material and Methods

Fifty healthy male pigeons without any signs of illness were obtained from a fancy commercial birds store and carried under the same condition to an appropriate place with temperature 21–3 °C and 12:12 hr light.
dark daily cycle. All birds were approximately in the same age (6-8 months) with mean weight of 300 g were caged in five groups randomly. Birds were kept in metallic cages of 1.8 (width) x 2.1 (length) x 0.5 (height) meter with wire-mesh on top, bottom and sides. Feed (wheat) and water were provided ad libitum.

Metoclopromide (0.5 mg/kg) (METOCLOPROMIDE, amp. 10 mg/2ml, DarouPakhsh, Tehran, Iran), atropine (0.04 mg/kg) (ATROPINE, amp. 0.5 mg/ml, DarouPakhsh, Tehran, Iran), midazolam (0.2 mg/kg) (MIDAMAX, amp. 5 mg/ml, TehranChemie, Tehran, Iran), acepromazine (0.1 mg/kg) (NEUROTRANQ, vial, 10mg/ml, Alfasan, Holland) and normal saline (0.5 ml, as control group) administered intramuscularly in each group.

Blood samples were taken before injection and also at 30 and 90 min after drug administration. PCV was determined at the moment using a microhaematocrit centrifuge (Labtron Co. Tehran, Iran) at 12000 g for 5 min. Serum cortisol concentration was found out with a commercial enzyme-linked immunosorbent assay (ELISA) kit (IBL, Hamburg, Germany) and an ELISA reader (Statfax, USA) used. Total protein (TP) was measured with Hitachi 717 (kit Randox, England). Number of leukocytes was estimated using hemacytometer and Natt and Herrick’s solution (Natt and Herrick 1952). Blood sugar (BS) measured by glucose oxidase method with standard commercial techniques (Pars Azmoon Co. Tehran, Iran) and automated analyses (Hitachi 717, Japan).

Effects of each drug on these factors were compared with other groups. Results were shown as means±SD. Statistical analysis was performed using PASW statistic version 18, one-way Analysis of Variance (ANOVA). Statements of statistical significance were based on P ≤ 0.05.

**Results**

The result of cortisol, packed cell volume, total protein, and blood sugar is given in Table 1. The cortisol concentration increased significantly in atropine treated pigeons after 30 and 60 minutes. No significant difference was found at three intervals in PCV and total protein between control and treated groups. Blood sugar was significantly high in acepromazine. The effect of different drugs on leuckocytes count and their ratio is shown in Table 2. Heterophils, lymphocytes and heterophil: lymphocyte (H:L) is given in Table 2. The result indicated that heterophil concentration increased significantly in atropine at 30 and 60 minutes while lymphocyte percentage decreased significantly.

**Discussion**

Hematologic response to stress situation varies among species. Stressful situations in mammals lead to release of adrenal glucocorticoids (Hardy et al. 2005) thus; serum glucocorticoid concentration would be an indicator of stress (Chrousos and Gold 1992).
In avian species, every kind of stress results in catecholamine release and therefore increased metabolism (Richard 1998; Moberg 1987; Cockrem and Silverin 2000). These hormonal changes can alter some...
clinical and paraclinical appearance that are considered as stress indicators (Marco et al. 1997; Moe and Bakken 1997; Davis and Maerz 2008).

In mammals, spleen contraction and consequent red blood cell release caused a raise in hematocrit after physical restraint. This effect of catecholamines does via alpha-adrenergic receptors and is lesser or absent in avian species (Lopez-Olvera et al. 2007).

Avian leukogram can be unpredictable between and within species. In some studies, biochemical and hematologic parameters were measured during stress condition. These reports show significant increase in RBC count, hematocrit and hemoglobin concentration (Hatting et al. 1988; Moe and Bakken 1997; Montané et al. 2003). There are significant differences of total WBC count between avian species. Because of quickly excitement of avian species following handling, heterophilic-leukocytosis and elevated heterophil:lymphocyte index (H/L ratio) observed, such as occurs in the present study. Traumatic injuries with local infection and most inflammation can also produce heterophilic leukocytosis. Chronic release of internal glucocorticoids can lead to a mild to moderate leukocytosis. Some reports show that glucocorticoids injection can cause lymphocytopenia and heterophilia in chickens. Another studies compared plasma corticosterone concentration and H/L ratio responses to various stressors and concluded that the H/L ratio is a better indicator of stress in poultry (Gross and Siegel 1983). Hematological manifestations of stress in avian species include changes in the number of circulating leukocytes in particular a pronounced heterophilia and lymphocytopenia which is a reliable indicator of stress (Maxwell 1993; Al-murani et al. 1997). Normal H/L ratio is about 0.4 but this can rise to 8 in birds under severe stress. According to Jensen et al., (2000), basophil numbers can also be increased during stress and this elevation can be more rapid than an increase in H/L ratio.

There are only slight unclear reports that include effect of anesthetics on hematologic parameters in avian species. In one study, effects of isoflurane anesthetic were measured on hematologic and plasma biochemical values of American Kestrels (Falco sparverius) which resulted in decrease of hematocrit, number of basophils and plasma proteins after 10 minutes of anesthesia (Dressen et al. 1999). Another study investigated effects of ketamine hydrochloride in Red-tailed Hawks and found no significant changes in hematologic parameters after 40 minutes of intramuscular injection (Kollias and McLeish 1978). Some reports showed mild leukocytosis and higher antibody secretion following tramadol or atropine-pethidine medication (Salo 1977; Sacerdote et al. 2000). Effects of opioids on WBCs were examined in another study. Leukocytosis and increased number of lymphocytes, increased antibody secretion and higher chemotaxis were listed as the effects of this drug family. In addition, natural killer cells activity ceased and lymphocytes responses rose during use of these drugs (Vella-Brincat and Macleod 2007).
Acepromazine has anticholinergic, antihistamine and antispasmodic effects and blocks alpha-adrenergic receptors that can be used for its neuroleptic feature in pre-anesthesia (Montane 2003). Acepromazine was reported as a vasodilator and could lower harmful effects of stress during physical restraint of animals (Lopez-Olvera et al., 2007). It has been reported to decrease erythrocyte count, hemoglobin concentration, PCV, lymphocytes count, monocytes and band neutrophils in stressed animals (Montane 2003; Lopez-Olvera et al. 2007). Acepromazine showed its effect by suppressing pituitary-adrenal axis and as a result, blocks secretion of adrenocorticotropic hormone (Kothari et al. 1961). However, in our study it seems that in acepromazine injection group, cortisol concentration, showed no significant changes in comparison with basic level. In the other hand, this drug has the ability of maintaining basic cortisol level during surgery procedure.

Midazolam also inhibited increasing of cortisol level as we observed in present experiment. This theory supported by the fact that it enhances the effect of the neurotransmitter GABA on the GABA_A receptors and lead to neural inhibition and sedation (Olkkola and Ahonen, 2008). It seemed that in our study strong sedation of the drug neutralized influence of epinephrine. Midazolam achieves peak of its effect about 30 minutes after intramuscular injection which can make a drop in cortisol level at the moment. This led to muscle relaxation and reduces wing movement. 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). Sedative effects of metoclopromide and atropine are still unknown. Atropine, an analogous to glucocorticoids, was widely evaluated by scientists (Kamphuis et al. 2002). Atropine is an anticholinergic alkaloid that blocks acetylcholine effect on sino-atrial and atrio-ventricular nodes, thus, results in tachycardia. In our study; atropine administration caused an increase in cortisol level. Because of sympathomimetic mode of atropine, higher level of H/L index was also seen with atropine more than other drugs. However, mild leukocytosis and the increase of secreted antibodies from B cell have been reported after atropine injection (Sacerdote et al. 2000). Atropine as a glucocorticoid can mobilize glucose and fatty acids for energy supply and provide better perfusion of the vital organs. Higher blood glucose concentration relies on internal glucocorticoids and indicates that all of these drugs are unable to decrease blood glucose concentration in the short time period. The avian spleen is considered to have little capacity for blood storage; therefore, released epinephrine in stress situation, cannot increase PCV. Because of the short-term study, total protein and therefore albumin and globulins remain unchanged.

**Conclusion**
Comparison between groups suggests that acepromazine and midazolam are more sedative than other drugs. It was concluded that midazolam is a powerful sedative drug that can reduce cortisol level. However, acepromazine has the ability of maintaining cortisol level during stress. More studies in birds are needed to find out drug of choice in each situation.

References