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Effects of the dexmedetomidine, midazolam, butorphanol, and atropine combination on plasma oxidative status and cardiorespiratory parameters in raccoon dogs (*Nyctereutes procyonoides*)

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ABSTRACT: Oxidative stress in the body occurs when the production of free radicals overwhelms the antioxidant defence systems. This study aimed to investigate the effects of a combination of dexmedetomidine, midazolam, butorphanol, and atropine (DMBA) as an anaesthetic on plasma oxidative status in twelve raccoon dogs. Baseline measures were recorded prior to anaesthesia, and then the animals were anaesthetised with the combination of dexmedetomidine (25 μg/kg), midazolam (0.45 mg/kg), butorphanol (0.25 mg/kg), and atropine (0.035 mg/kg). Temperature, respiratory rate, haemoglobin saturation by oxygen, pulse rate, systolic arterial pressure, diastolic arterial pressure, and mean arterial pressure were continually monitored. Blood pressure was significantly decreased at 30 to 60 min ($P < 0.05$). Pulse rate ranged from 96 to 123 bpm, without episodes of severe bradycardia or tachycardia. Blood samples were collected from saphenous venipuncture at 0, 0.5, 1, and 24 h before, during, and after anaesthesia. Plasma superoxide dismutase, glutathione peroxidase, and catalase activity, and malondialdehyde concentrations were measured by colorimetry, and plasma vitamin E level was determined by high-performance liquid chromatography. Superoxide dismutase and glutathione peroxidase activities increased significantly ($P < 0.05$) at 0.5 h, and then gradually decreased to baseline values after 1 h. Catalase activity increased significantly ($P < 0.05$) at 0.5 h, 1 h, and then gradually decreased to baseline values after 24 h. There was no significant change in vitamin E level ($P < 0.05$).The concentration of malondialdehyde decreased significantly at 0.5, 1, and 24 h after injection ($P < 0.05$). The results show that the administered dose of dexmedetomidine, midazolam, butorphanol, and atropine has antioxidant effects in raccoon dogs. Our study is the first to demonstrate that dexmedetomidine, midazolam, butorphanol, and atropine exert antioxidant effects, which may be exploited to alleviate the stress of examination and research at veterinary clinics.

Keywords: anaesthesia; lipid peroxidation; oxidative stress; DMBA

The production of reactive oxygen species (ROS) in low concentrations is absolutely necessary for physiological processes such as cell differentiation and proliferation, apoptosis, and cell-mediated immunity (Simeonova et al. 2004). Many exogenous factors such as increased or decreased partial oxygen pressure in blood, chemicals and drugs may cause an increase in ROS formation (Hug et al. 1997). Enzymatic and non-enzymatic antioxidant defences maintain ROS concentrations in the physiological range. Antioxidants are generally divided into enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic (vitamins C and E) categories (Zhang and Zehnder 2013). Oxidative stress in the body occurs when the production of free radicals overwhelms the antioxidant defence systems, resulting in damage of DNA and certain membrane lipids (Halliwell 1994). The mechanisms of action of antioxidants may involve limiting the formation of free radicals through inhibition of lipid peroxidation and protein oxidation or the neutralisation of their toxic effects (Little and Gladen 1999). The pro-oxidation effects of some general anaesthetics have been described (Khinev

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et al. 1994), and various anaesthetics possessing different physicochemical properties have been reported to affect the lipid peroxidation process directly or indirectly, leading to the formation of malondialdehyde (MDA) in the body or even to tissue damage (Yaralioglu-Gurgoze et al. 2005).

Dexmedetomidine is a potent and selective agonist of the α_2-adrenoceptor. Desirable properties of dexmedetomidine include induction of sedation and analgesia via stimulation of α_2-receptors without concomitant respiratory depression (Gerlach et al. 2009). Common side effects of dexmedetomidine include bradycardia and hypothermia. Midazolam, a benzodiazepine analgesic, exerts amnestic, sedative, hypnotic, anxiolytic, and anticonvulsant properties (Wright et al. 1990). In addition, midazolam holds the advantages of promoting a rapid onset of anaesthesia and of resulting in only minimal cardiovascular and respiratory effects (Fragen 1997). Butorphanol is an opioid agonist-antagonist and, as such, is a competitive antagonist at the μ receptor and an agonist at the κ receptor. This mixed agonistic-antagonist activity results in analgesia and is less likely to induce respiratory depression compared with pure μ agonistic activity (Portnoy and Hustead 1992; Gillis et al. 1995). This activity is a useful characteristic in the raccoon dog (*Nyctereutes procyonoides*) because its economic value indicates the need for relatively safe treatment. Butorphanol is most commonly used to control mild to moderate pain in small animals and moderate visceral pain in horses (Papich 1990). Atropine is a competitive antagonist for the muscarinic acetylcholine receptors and is classified as an anticholinergic drug (Shen et al. 2014). Atropine prevents dexmedetomidine-induced bradycardia.

Although there have been many studies on the effects of different anaesthetics on antioxidants, lipid peroxidation, and biochemical and cardiorespiratory parameter changes in different species, to our knowledge there has been no work reporting the effect of dexmedetomidine, midazolam, butorphanol, and atropine in combination (DMBA) on plasma glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) activities and lipid peroxidation in raccoon dogs. In the present study, we aimed to investigate the effect of DMBA on plasma GSH-Px, CAT and SOD activities, plasma MDA and VE (vitamin E) concentrations, and cardiorespiratory parameters before, during, and after anaesthesia in the raccoon dog.

MATERIAL AND METHODS

Animals and drugs. Twelve adult raccoon dogs with a good nutritional status, eight months old, and weighing (mean ± SD) 9.02 ± 0.37 kg, (six males and six females) were selected from a breeding colony in Shengyuan of Heilongjiang Province. The study was approved by the Northeast Agricultural University Committee on Institutional Animal Care and Use in China. All animals were determined to be healthy based on the results of a complete physical examination. The raccoon dogs were manually restrained and no sedatives or anaesthetics were used. Food, but not water, was withheld for 12 h prior to anaesthesia. All of the raccoon dogs underwent a period of acclimation 48 h before the study. Animals were anesthetised using a hand held syringe with 25 ug/kg dexmedetomidine (Pfizer Animal Health, NY, USA), 0.45 mg/kg midazolam (USP; Hospira, Inc., IL, USA), 0.25 mg/kg butorphanol (Sigma Chemical Co, St. Louis, MO), and 0.035 mg/kg atropine (Grunenthal GmbH, Aachen, Germany) intramuscularly. Cardiorespiratory parameters were monitored. The raccoon dogs were placed in lateral recumbence, breathing air spontaneously without intubation. Anaesthesia was established within 3.9 min (range 2.6–5.1 min) and lasted approximately 1.3 h (mean, 79.22 ± 12.19 min).

Cardiorespiratory parameter monitoring. Monitoring of anaesthesia, as well as body temperature (T), respiratory rate (RR), pulse rate (PR), haemoglobin saturation by oxygen (S_pO_2), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP), was performed. These variables were recorded at 5, 10, 20, 30, 40, 50, and 60 min following administration of drugs. Body temperature was measured with a standard rectal thermometer in the anus. Pulse rate (PR) and haemoglobin saturation by oxygen (S_pO_2) were measured with a pulse oximeter clipped on the tongue of the raccoon dog. Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) were measured using cuffs wrapped around limbs. Respiratory rate (RR) was visually determined by counting movements of the thorax.

Blood sampling. A volume of 2 ml of blood was collected by left or right saphenous venipuncture into a Vacutainer® containing EDTA before (0 h; baseline) and at 0.5, 1, and 24 h after administration of drugs. The samples were immediately centrifuged at 1500 × g for 15 min, and plasma samples

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were extracted. Samples were stored at –80 °C until further analysis of MDA levels, and GSH-Px, superoxide dismutase (SOD), and catalase (CAT) activities. The results were determined using commercially available assay kits (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). Plasma vitamin E level was determined by high-performance liquid chromatography (HPLC) using a 4.6 mm × 25 cm C-18 type column (Waters Symmetry) and a 4 mm × 1 cm pre-column, at a flow rate of 2.0 ml/min (Vannucchi et al. 2007).

Statistical analysis. Statistical analysis was performed using SPSS Version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Changes in values following administration of DMBA were compared with baseline values using one-way ANOVA. Data are expressed as mean ±SD. $P < 0.05$ was considered as significant.

RESULTS

Cardiorespiratory parameter changes

All raccoon dogs recovered from anaesthesia without complications and no excitement was observed during anaesthesia. Body temperature was found to drop by 1.0 °C after administration of drugs. Respiratory rate (RR) and haemoglobin saturation by oxygen (S_pO_2) increase slightly during anaesthesia but this was not significant. Pulse rate (PR) decreased significantly ($P < 0.05$) during anaesthesia compared with the 5 min time point. Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) decreased significantly from 30 to 60 min ($P < 0.05$) during anaesthesia compared with the 5 min time point (Table 1).

Changes in oxidative stress indicators

The activities of SOD and GSH-Px were significantly higher at 0.5 h ($P < 0.05$) compared with baseline values. The activity of CAT was significantly increased at 0.5 and 1 h compared with baseline values ($P < 0.05$). Although vitamin E levels were slightly increased during anaesthesia, this was not significant ($P > 0.05$). The concentrations of MDA were significantly decreased at 0.5 h, 1 h and 24 h compared with baseline values ($P < 0.05$, Table. 2).

DISCUSSION

Mild hypothermia is extremely common during anaesthesia and surgery. The basic process occurs as core body heat redistributes to the skin surface through anaesthetic-induced vasodilation and depression of hypothalamic thermoregulatory centres. Schroeder and Smith (2011) indicated that midazolam-butorphanol combinations resulted in decreased body temperatures in rabbits. In our study, body temperature initially decreased during anaesthesia, but remained above 38 °C, so hypothermia did not occur. S_pO_2 did not vary and was above 95% throughout the period of anaesthesia. RR did not change throughout the period of anaesthesia.

Dexmedetomidine has been used successfully in a variety of clinical settings to produce sedation, analgesia, and anaesthesia (Mantz et al. 2011;

Table 1. Changes in physiological parameters at different time points during anaesthesia

Time (min)	T (°C)	RR (breaths/min)	PR (beats/min)	S_pO_2 (%)	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)
5	38.6 ± 0.3	17.7 ± 5.3	123.8 ± 7.1	95.8 ± 1.8	149.0 ± 8.6	96.5 ± 6.9	113.8 ± 7.2
10	37.6 ± 0.3*	20.0 ± 5.2	113.1 ± 6.6*	96.7 ± 1.0	136.5 ± 12.8	92.3 ± 10.6	107.1 ± 6.4
20	38.1 ± 0.3*	20.3 ± 5.8	111.5 ± 9.6*	96.5 ± 1.0	134.5 ± 11.7	87.2 ± 6.9	102.7 ± 7.1*
30	38.3 ± 0.4	18.8 ± 5.3	109.2 ± 5.8*	96.8 ± 0.8	127.7 ± 15.3*	84.5 ± 7.4*	98.8 ± 8.5*
40	38.3 ± 0.2	17.8 ± 4.5	99.0 ± 5.1*	96.5 ± 1.5	126.1 ± 9.3*	84.5 ± 10.6*	98.3 ± 6.5*
50	38.3 ± 0.3	18.0 ± 6.1	95.5 ± 5.2*	97.0 ± 1.3	130.3 ± 9.7*	85.8 ± 6.6*	100.5 ± 7.7*
60	38.4 ± 0.4	17.5 ± 4.5	111.7 ± 10.2*	97.2 ± 1.2	127.8 ± 20.1*	82.8 ± 9.5*	97.5 ± 11.9*

Values are mean ± SD; T = temperature; RR = respiratory rate; HR = heart rate; SpO_2 = blood oxygen saturation; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure

*$P < 0.05$ compared with 5 min within each group

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Table 2. Changes in oxidant/antioxidant parameters before and after anaesthesia in raccoon dogs

Times (h)	SOD (IU/ml)	GSH-Px (IU/ml)	VE (mg/ml)	MDA (nmol/ml)	CAT (IU/ml)
0	113.05 ± 11.38	396.01 ± 39.30	2.81 ± 0.28	12.69 ± 3.56	2.71 ± 0.39
0.5	151.87 ± 17.64*	563.40 ± 76.73*	2.76 ± 0.31	9.09 ± 1.44*	3.49 ± 0.68*
1	124.41 ± 10.90	445.28 ± 39.50	3.09 ± 0.25	7.98 ± 1.23*	3.57 ± 0.84*
24	107.98 ± 8.49	387.86 ± 51.32	3.13 ± 0.51	6.50 ± 093*	3.11 ± 0.59

Values are mean ± SD; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; MDA = malondialdehyde; CAT = catalase

*$P < 0.05$ compared with 0 h within each group

Murthy and Singh 2009). The most common effect of highly selective α_2-agonists, such as dexmedetomidine, is baroreceptor-induced hypertension and subsequent normotension or mild hypotension (Murrell and Hellebrekers 2005). In raccoon dogs anaesthetised with the DMBA combination, the pulse rate and blood pressure were changed after drug administration. This is likely due to the fact that midazolam decreases systemic vascular resistance (Jones et al. 1979; Samuelson et al. 1981) which is often accompanied by compensatory decreases in PR. Kunisawa et al. (2009) reported that administration of dexmedetomidine in combination with other anaesthetics during anaesthetic induction may prevent a decrease in blood pressure. The results of our study suggest that the effects of dexmedetomidine probably temporarily and partially counterbalance the hypotension effect of midazolam at 5, 10, and 20 min after anaesthesia. Our results also show a significant decrease in SAP, DAP, and MAP at 30 to 60 min anaesthesia compared with 5 min after anaesthesia, but these values are still within the normal range of blood pressure values. Midazolam-induced reductions in systemic vascular resistance may be responsible for these effects.

The release of free radicals during oxidative stress causes severe damage to organisms by removing electrons from macromolecules causing instability and disintegration. Antioxidants, in general, are compounds and reactions which dispose of, scavenge, and suppress the formation of ROS (Valko et al. 2007). The main enzymes that control the biological effects of reactive oxygen species are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). SOD catalyses the dismutation of superoxide anions into H_2O_2, while glutathione peroxidase (GPX) detoxifies H_2O_2 and lipid peroxides (Zelko et al. 2002; Jurkovic et al. 2008). Catalase (CAT) acts in the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. These antioxidant enzymes respond to increased oxidative stress and act as scavengers of reactive oxygen species. Therefore, measuring the activity of antioxidant enzymes reflects the antioxidative status of the antioxidant defence system. Naziroglu and Gunay (1999) reported that serum GSH-Px activity did not change significantly during anaesthesia, possibly because it was significantly lower than in erythrocytes and other tissues. In contrast, Neri et al. (1993) observed an increase in GSH-Px activity during anaesthesia. Our results also show a significant increase in GSH-Px activity. Vitamin E is a potent protector against the formation of free radicals. In our study, levels of VE did not change, perhaps, because the levels of this vitamin were already sufficiently high in the organism.

In vivo, the role of reactive oxygen species is to induce peroxidation, and the end product of lipid oxidation is MDA. Thus, malondialdehyde (MDA) content reflects the degree of lipid peroxidation in an organism (Ardestani and Yazdanparast 2007). Khinev et al. (1994) reported that there is no significant change in MDA levels before and after anaesthesia in humans. However, other studies have reported increased serum MDA levels during anaesthesia in dogs (Naziroglu and Gunay 1999) and also in humans (Neri et al. 1993; Glantzounis et al. 2001). Our study showed a decrease in plasma MDA levels during anaesthesia compared with values prior to anaesthesia. This finding indicates that the degree of lipid peroxidation is decreased during anaesthesia. Our study showed that raccoon dogs injected with DMBA had increased plasma SOD, GSH-Px and CAT activity. This increase in activity could act to maintain the oxidant/antioxidant balance, indicating that DMBA is capable of increasing antioxidant capacity in the raccoon dog.

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In conclusion, the combination of DMBA causes transient oxidative stress in the raccoon dog, which may then lead to an increase in GSH-Px, SOD, CAT activities. Our results support the hypothesis that the use of anaesthetics such as DMBA causes oxidative stress in the raccoon dog. Therefore, anaesthetics should be used cautiously in the veterinary clinic and in research.

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