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Oxidative stress response of guaifenesin-ketamine anaesthetized cattle during surgery under detomidine/xylazine premedication

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Abstract

The study was conducted in twelve clinical cases of cattle undergoing diagnostic and surgical procedures warranting general anaesthesia. The cases were randomly divided into two groups: group I and group II each consisting of six cases. Detomidine @ 30 µg/kg (0.03 mg/kg) body weight and Xylazine @ 0.1 mg/kg body weight were used as pre-anaesthetics in groups I and II respectively. Ketamine @ 2.0 mg/kg body weight and guaifenesin @ 50.0 mg/ml of 5% dextrose solution was used as induction and maintenance agent in both the groups. There was no significant differences observed in the neutrophil, lymphocyte, eosinophil and monocyte counts in both the groups. The magnitude of blood glucose was higher in group II compared to that of group I. A non significant reduction in total serum protein was observed after sedation, after induction, during maintenance and after recovery in both the groups. Group II exhibited a significant increase in plasma cortisol level, whereas in group I, a reduction in plasma cortisol level noticed. Antioxidant activity was more marked in group II when compared to group I.

Keywords: Cattle, Oxidative stress, Detomidine, Guaifenesin, Ketamine, Xylazine, Antioxidants

Introduction

In general, cattle are not good subjects for general anesthesia. Anaesthetizing cattle is a challenging work as they are more prone for potential complications during anesthesia. Regurgitation and increased salivation under anaesthesia can cause aspiration. Eructation is impeded and gas accumulates causes bloating. Bloating can be severe if there is a large amount of ingesta in the stomach and/or if the anesthetic event is extended. Adult cattle carries greater risk of developing myopathies and neuropathies following prolonged recumbency. In addition, the weight of the abdominal viscera and their contents prevents the diaphragm from moving freely on inspiration and ventilation becomes shallow, rapid and inefficient for gas exchange within the lungs (Lee, 2006, Anderson, 2013 and Kaiser-Klingler, 2013).

The prime goals in cattle during anaesthesia and perioperative period should be to prevent the earlier mentioned risks, maintain normal cardiac output, blood pressure and acid-base balance, to ensure adequate ventilation and oxygenation and to minimize the factors responsible for triggering stress and release of oxygen free radicals (Wagner, 1991). Oxidative stress occurred when the production of oxygen free radicals (RSO = Reactive oxygen species) and nitric oxide radicals (NOR) exceeded the scavenging capacity of systemic endogenic antioxidants through enzymatic and non enzymatic pathways (Basu *et al*., 2001).

The reactive oxygen species were hydrogen peroxide, superoxide anion, peroxide radicals, alkoxy radical and peroxy radical. Reactive nitrogen species were nitric oxide and peroxynitrite (Brasil *et al*., 2006). The production of these free radicals was favoured directly by reduced perfusion and tissue anoxia and indirectly by anaesthetic and ancillary drugs (Basu *et al*., 2001 and Ozer and Kaman, 2007). Free radicals induce stress, inflammation, delay in wound healing, prolonged recovery from anaesthesia and post anaesthetic complications due to the impact on the functional ability of heart, lung, spleen, liver, kidney, red blood corpuscles and muscles (Brasil *et al*., 2006). Free radical also interferes with the regeneration of tissues by inducing single bond breakage of DNA in morphology of cells. Ketamine and guaifenesin are commonly used in cattle as the common induction agent and also useful for maintenance in the field of ambulatory bovine practice. Detomidine and xylazine were the common sedative agents used for premedication. The aim of the study was to assess the magnitude of physiological stress in terms of plasma cortisol and oxidative stress in terms of enzymatic and non enzymatic antioxidants. This assay will reflect on the quantum of oxygen free radical in a directly proportional manner.

Materials and Methods

The study was conducted on 12 cattle reported to large animal surgery operation theatre of Madras Veterinary College Teaching Hospital. The selected cattle were randomly allotted to either group I or group II consisting of 6 cattle each. Feed and water were withheld for 18 hours and 6 hours respectively prior to anaesthesia on the day of procedure. Jugular vein was cannulated in all the cattle. Group I cattle were premedicated with detomidine and group II cattle were premedicated with xylazine hydrochloride. At peak sedation, ketamine-guaifenesin mixture was administered intravenously to attain induction and maintenance of anaesthesia to complete the surgical procedure in both the groups.

Blood samples were collected before sedation, after sedation, after induction, during maintenance and after recovery in both the groups for plasma cortisol and antioxidant estimation. The enzymatic and non enzymatic antioxidants estimated were Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px), Catalase(CAT) and Reduced Glutathione (GSH).

Heparinized blood samples were centrifuged (1500rpm, 10 min) and supernatants were immediately stored at -80°C until analysis. Plasma cortisol was measured quantitatively using competitive Enzyme Linked Immuno Sorbent Assay - ElAgen Cortisol Kit. The activity of superoxide dismutase (SOD) was determined using the method of Marklund and Marklund (1974). The assay is based on the ability of SOD to exhibit the autooxidation of pyrogallol in the presence of EDTA. The values were expressed as Units/mg Hb. The plasma glutathione peroxidase (GSH-Px) activity was determined according to the method of Hafeman *et al*. (1974). The rate of oxidation of GSH by H_2O_2 was used as measure of GSH-Px activity and expressed as Units/mg Hb. The plasma catalase (CAT) activity was measured as per the method described by Aebi (1983). 20 µl of 1% erythrocyte lysate was incubated in 1.0 ml of 30 mM H_2O_2 at 37°C and decrease in absorbance was noted at every 10 sec interval for one min. at 240 nm in a UV spectrophotometer (Schimadzu UV-1208 UV-VIS, Japan). 1unit catalase activity was defined as the amount of enzyme that decomposes 1µM of H_2O_2/min. and expressed as Units⁄mg Hb. The glutathione level in plasma was measured by the method of Moron *et al*. (1979). This method was based on the reaction of reduced glutathione with 5-5 dithiobis-2-nitro-benzoic acid (DTNB) to produce a compound that absorbs light at 412 nm. The values are expressed as µgm/ml plasma.

The data were analysed using Statistical Package for Social Sciences (SPSS - 19).

Results and Discussion

Plasma cortisol

The mean plasma cortisol level in ng per ml ranged between7.00 ± 1.47 and 8.93 ± 0.73 in group I and 9.33 ± 0.70 and 105.53 ± 1.40 in group II following different stages of study (Table 1). Statistical analysis revealed a highly significant increase in the plasma cortisol level after sedation, after induction and during maintenance in group II, whereas in group I, a non significant reduction noticed during the period of study. After recovery the values started to move towards the base value.

Increase in plasma cortisol level following surgical trauma and anaesthesia was reported by Clarke *et al*. (1970) and the reasons attributed were decreased breakdown of plasma cortisol due to reduction in hepatic blood flow during surgery and elevation of plasma half life of cortisol. Traynor and Hall (1981) and Davis (1990) attributed elevated plasma cortisol level to the activation of stress response following impulses arising from afferent nerve fiber both systemic and autonomic from the

surgical wound and by release of humoral factors such as prostaglandin, histamine, kinins and leukotriens. Correlation of these diverse signals occurred in the hypothalamus.

Table 1. Mean ± SE value of Plasma cortisol in group I and group II

Parameters	Group	Before sedation	After sedation	After induction	During maintenance	After recovery
Plasma cortisol (ng/ml)	I	8.93 ± 0.73	7.73 ± 1.09	7.13 ± 1.12	7.00 ± 1.47	8.42 ± 1.11
	II	9.33^{a} ± 0.70	79.60^{c}±0.95	94.53^{d}±1.33	105.53^{e}±1.40	64.87^{b}±1.12

Means bearing different superscripts in a row differ significantly (P<0.05)

In the present study, elevation of plasma cortisol level following administration of xylazine as premedicant could be attributed to its alpha$_2$ adrenergic agonistic action (Bettschart-Wolfensberger *et al.,* 1996). Detomidine did not influence the cortisol level in horses (Raekallio *et al*., 1991) and there was a significant reduction in cortisol level in horses after detomidine administration due to reduction in sympatho-adrenal activity (Raekallio *et al*., 1992). ketamine by its sympathotonic effect and activation of pituitary adrenocortical axis stimulated the plasma cortisol level (Bettschart-Wolfensberger *et al*., 1996 and Mahalingam *et al*., 2014). Taylor and Watkins (1992) , Taylor *et al*. (1995), Luna *et al*. (1996) and Taylor *et al*. (1998) found out a reduction in plasma cortisol concentration during detomidine, ketamine, guaifenesin anaesthesia.

It could be concluded that detomidine premedication induced less stress in terms of protein level, blood glucose level, percentage of neutrophils, lymphocyte, eosinophil, monocyte and plasma cortisol level.

Antioxidants

The mean SOD activity in Units per milligram Hb ranged between 6.24 ± 0.26 and 6.72 ± 0.28 in group I and 5.81 ± 0.32 and 6.40 ± 0.40 in group II following different stages of study (Table 2). Statistical analysis revealed nonsignificant difference between means of the group I and group II at different stages of the study. However, the magnitude of SOD enzyme activity was less in group I when compared with group II.

The mean GPx enzyme activity in Units per milligram Hb in group I ranged between 1.93 ± 0.09 and 2.19 ± 0.06, while in group II it ranged between 2.24 ± 0.08 and 2.62 ± 0.16 (Table 2). Statistical analysis revealed significant increased activity of GPx in both the groups. However, the activity of GPx was less in group I when compared with group II.

Table 2. Mean ± SE value of antioxidants in group I and group II

Parameters	Group	Before sedation	After sedation	After induction	During maintenance	After recovery	Summation of activity
Super oxide disamutase (U/mg Hb)	I	6.24^{a} ± 0.26	6.36^{b} ± 0.21	6.56^{c} ± 0.34	6.72^{d} ± 0.28	6.31^{ab} ± 0.29	32.19
	II	5.81^{a} ± 0.32	5.98^{b} ± 0.22	6.20^{c} ± 0.46	6.40^{d} ± 0.40	5.85^{a} ± 0.31	30.24
Glutathione peroxidase (U/mg Hb)	I	1.93^{a} ± 0.09	1.99^{b} ± 0.09	2.10^{c} ± 0.07	2.19^{d} ± 0.06	2.05^{c} ± 0.15	10.26
	II	2.24^{a} ± 0.08	2.34^{b} ± 0.11	2.48^{c} ± 0.13	2.62^{d} ± 0.16	2.36^{b} ± 0.09	12.04
Catalase (U/mg Hb)	I	42.34^{a} ± 1.84	44.01^{b} ± 1.61	46.41^{c} ± 1.32	47.89^{d} ± 1.94	44.72^{b} ± 1.73	225.37
	II	38.97^{a} ± 1.02	41.81^{c} ± 1.09	44.06^{d} ± 1.11	45.62^{e} ± 1.34	40.21^{b} ± 1.54	210.67
Reduced glutathione (µg/ml plasma)	I	71.81^{d} ± 2.99	74.93^{e} ± 2.05	68.11^{c} ± 2.43	66.72^{b} ± 2.48	63.34^{a} ± 2.83	344.91
	II	76.09^{d} ± 2.39	79.62^{e} ± 3.01	74.84^{c} ± 2.29	66.72^{a} ± 2.76	70.84^{b} ± 2.90	368.11

Means bearing different superscripts in a row differ significantly (P<0.05)

The mean CAT enzyme activity in Units per milligram Hb ranged between 42.34 ± 1.84 and 47.89 ± 1.94 in group I and 38.97 ± 1.02 and 45.62 ± 1.34 in group II during the period of study (Table 2). Statistical analysis revealed significant increase in catalase enzyme activity in both the groups during the period of study. However, the activity of catalase was higher in group II when compared with group I.

The mean GSH activity in microgram per ml plasma in group I ranged between 63.34 ± 2.83 and 74.93 ± 2.05 and 66.72 ± 2.76 and 79.62 ± 3.01in group II during the period of study (Table 2). Statistical analysis revealed significant increased activity of GSH in both the groups after sedation. The activity of non enzymatic antioxidant GSH was less in group I when compared with group II.

Oxidative stress occurred when the production of oxygen free radicals (RSO = reactive oxygen species) and nitric oxide radicals (NOR) exceeded the scavenging capacity of systemic endogenic antioxidants through enzymatic and non enzymatic pathways (Basu *et al*., 2001). The reactive oxygen species were hydrogen peroxide, superoxide anion, peroxide radicals, alkoxy radical and peroxy radical. Reactive nitrogen species were nitric oxide and peroxynitrite (Brasil *et al*., 2006). The production of these free radicals was favoured directly by reduced perfusion and tissue anoxia and indirectly by anaesthetic and ancillary drugs (Basu *et al*., 2001 and Ozer and Kaman, 2007). The free radicals were released from cytochrome P450, endothelium, granulocytes, macrophages and red blood corpuscles. Free radicals caused stress, inflammation, delay in wound healing, prolonged recovery from anaesthesia and post anaesthetic complications due to the insult on the functional ability of heart, lung, spleen, liver, kidney, red blood corpuscles and muscles (Brasil *et al*., 2006). The half- life of free radicals is 10^{-6} to 10^{-9} seconds at 37^0C except hydrogen peroxide; hence the level of free radicals was indirectly assessed by the directly proportional activity of enzymatic antioxidants namely SOD, GPx and CAT and non enzymatic antioxidants - GSH (Delogu *et al*., 2004).

In the present study, the sum of total activity of antioxidants namely SOD, GPx, CAT and GSH increased after premedication with xylazine/detomidine and further progressively increased after induction and during maintenance with ketamine - guaifenesin. The activity started declining after recovery. Between the groups, antioxidants activity was less when detomidine was used as premedication agent. Xylazine reduced tissue perfusion (Steffey *et al*., 1977 and Wagner *et al*., 1991) and depended on hepatic cytochrome P450 for metabolism leading to elevated free radical formation which resulted in high antioxidant activity after premedication. Detomidine was not lowered tissue perfusion (Taylor *et al*., 1998) hence less free radical formation than xylazine.

Ketamine due to increase in sympathetic tone increased the vascular resistance leading to tissue anoxia and release of free radicals. As ketamine depended on hepatic cytochrome P450 for detoxification and biotransformation (except 4 per cent excreted as whole), the release of free radicals was more which inturn resulted in higher antioxidant activity. Alva *et al*. (2006) reported that xylazine - ketamine induced oxidative stress without risking hepatic toxicity by increasing the level of plasmatic nitric oxide and change in acid-base balance and metabolic acidosis. The result of the present study was supported by the findings of Mahalingam *et al*. (2014), as the authors reported that the xylazine - ketamine anaesthesia in dogs increased the lipid peroxidation. This was accompanied by enhanced antioxidant status as the superoxide dismutase and catalase which provided the first line of defence against the reactive oxygen species induced damages. The increased activity of superoxide dismutase dismutases the superoxides and resulted in H_2O_2 generation, which is decomposed by catalase into H_2O and O_2. Feng *et al*. (2015) found an increase in superoxide dismutase, glutathione peroxidase and catalase level during anaesthesia due to the activation of antioxidant mechanism to prevent the oxidative stress. The results of the present study was supported by the findings of Bisla *et al*. (2004), Serin *et al*. (2008) and Sankar *et al*. (2010).

Conclusion

The plasma cortisol level significantly elevated in xylazine premedicated group cattle, whereas in detomidine premedicated group the plasma cortisol level was reduced. The antioxidant level was significantly higher in xylazine premedication when compared to detomidine premedication, revealing higher production of oxygen free radicals due to less tissue perfusion and activation of hepatic cytochrome P450. It was concluded that detomidine premedication in ketamine-guaifenesin anaesthesia induces less physiological stress in terms of plasma cortisol level and oxydative stress in terms of enzymatic and non enzymatic antioxidants than xylazine premedication in cattle. Hence detomidine can be a better agent in cattle for sedation as well as for premedication.

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