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LIPID PEROXIDATION IN HAEMORRHAGIC SHOCK AND AFTER TRANSFUSION OF BLOOD IN DOGS

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Abstract : The present study was carried out on mongrel dogs. Haemorrhagic shock of different severities and duration was produced by exsanguination from an artery. After the required duration of shock, two third of the volume of blood withdrawn was transfused back into the animal. Effect of haemorrhage and reperfusion of blood after haemorrhagic shock on lipid peroxidation was assessed by measuring plasma malondialdehyde (MDA). Severity of shock was assessed from the haematocrit values. There was a significant increase ($P<0.05$) in plasma MDA level after blood transfusion in a group having 40mm Hg blood pressure as magnitude of shock and one hour as duration of shock (Group II) only. Haemotocrit value was also significantly low ($P<0.05$) in this group after haemorrhagic shock. Results are suggestive of lipid peroxidation with ischaemic reperfusion in severe and long duration of shock.

Key words: haemorrhagic shock reperfusion plasma MDA haematocrit reperfusion injury

INTRODUCTION

Haemorrhagic shock is life-threatening situation. Reflex sympathetic vasoconstriction impairs blood flow to skin, splanchnic and renal areas to favour the blood flow in coronary and cerebral arteries (1). Impairment of microcirculation results in cellular damage and ischaemic necrosis of intestine due to excessive vasoconstriction of intestine(2). Various reports suggesting the role of free radicals in causing ischaemic-reperfusion injury on isolated organs like heart (3, 4), brain (5) and kidney (6) are available.

Meerson et al (7) reported that free radicals produced due to ischaemic

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reperfusion have got deleterious effects on various organs through formation of lipid peroxides. Polyunsaturated fatty acids present in cell membranes are affected which lead to increased fluidity, permeability and loss of membrane integrity. Peroxidation of unsaturated fatty acids cause the production of aldehydes like malondialdehyde (MDA). Haemorrhagic shock which causes generalised ischaemia of skin and splanchnic area and transfusion of blood/fluid to reconstitute the blood volume as part of treatment of shock can be viewed as ischaemic-reperfusion.

Keeping these in mind, the present study was undertaken to determine the levels of plasma malondialdehyde which is considered as an index of lipid peroxidation and haematocrit to know severity of shock in dogs after producing haemorrhagic shock and after reperfusion of blood and tried to correlate the extent of lipid peroxidation with the severity of ischaemic and reperfusion injuries. This may help in the understanding of pathophysiology of irreversible shock and bringing changes in the line of treatment.

METHOD

Twenty mongrel dogs of either sex, weighing 10-15Kg. were used. They were divided into four groups of five dogs each.

Group I B.P. maintained at 40mm Hg for ½ hour

Group II B.P. maintained at 40mm Hg for 1 hour

Group III B.P. maintained at 60mm Hg for ½ hour

Group IV B.P. maintained at 60mm Hg for 1 hour

Anaesthesia was induced with Thiopentone sodium (Pentothal 30mg/kg. body weight intravenously) and maintained by supplemental intraperitoneal doses of the anaesthetic administered as and when required. Dogs were heparinised by 1000 i,u/kg. body weight intravenously. Haemorrhage was produced by drawing blood from the femoral artery at the rate of 0.5 to 1 ml/kg/min and collecting it in a pre-heparinised container. Blood pressure was maintained at 40 or 60 mm Hg as per the group by withdrawing or transfusing the blood through femoral vein as and when required. After maintaining the haemorrhagic shock for the required period, two third of the withdrawn blood was transfused through the femoral vein. Venous blood samples were collected before hemorrhage, after maintaining shock, ½hr, 1hr and 2 hrs after blood transfusion and analysed for haematocrit values by microcapillary method (8) and plasma MDA by thiobarbituric acid (TBA) assay method (9). These were compared to the values obtained in the blood sample of same animal drawn after anaesthesia but just before producing haemorrhagic shock (which served as group control). Paired t-test was used to assess the significance of results.

RESULTS

Table I shows the effect of haemorrhagic shock of different severity and duration (according to groups) on plasma MDA. There is increase in Plasma MDA, after maintainance of shock as compared to controls in all the four groups though the difference is not significant statistically.

TABLE I: Plasma MDA (nmol/ml) levels in mongrel dogs before & after haemorrhagic shock. (mean ± SE).

Group	*Before haemorrhagic shock (Control)*	*After maintaining haemorrhagic shock*
I	2.004±0.592	2.176±0.707
II	2.192±0.460	3.078±0.751
III	2.490±0.795	2.782±1.070
IV	1.244±0.168	1.948±0.673

Table II shows the effect of blood transfusion given to the various haemorrhagic shock groups on plasma MDA levels after 30 min, one hour and two hours of transfusion. Plasma MDA is increased significantly ($P<0.05$) in Group II after 1 and 2 hrs of blood transfusion as compared to control. In all other groups the rise is there but not significant.

Table III shows haematocrit values in controls (before producing haemorrhagic shock), after maintainance of shock and after transfusion of blood in various groups. There is significant decrease ($P<0.05$) in haematocrit value in Group II after haemorrhagic shock which is of severe extent (40 mm Hg) and longer duration (1 hr).

TABLE II: Plasma MDA (nmol/ml) levels in mongrel dogs in control and after transfusion of blood. (mean ± SE).

Group	*Before haemorrhagic shock (Control)*	*After transfusion of blood*		
		½ hr	*1 hr*	*2 hr*
I	2.004±0.592	2.408±0.947	2.604±1.234	3.336±1.208
II	2.192±0.460	3.130±0.719	3.506±0.328*	3.872±0.600*
III	2.490±0.795	2.398±0.754	2.788±0.724	3.156±0.728
IV	1.244±0.168	1.324±0.308	1.672±0.285	2.012±0.413

*P<0.05 as a compared to control

TABLE III: Haematocrit values (%) in mongrel dogs before & after haemorrhagic shock and after transfusion of blood. (mean ± SE).

Group	*(Control)*	*After maintaining haemorrhagic shock*	*After transfusion of blood*		
			½ hr	*1 hr*	*2 hr*
I	42.2±3.0	40.1±3.6	41.8±3.6	40.6±3.7	40.2±3.5
II	39.4±2.2	37.2±2.6*	38.4±2.0	39.4±1.7	38.8±2.0
III	42.0±3.20	40.0±3.3	42.4±3.7	42.0±3.4	42.0±3.6
IV	41.2±3.6	40.4±2.7	41.2±2.9	40.0±2.8	40.2±2.3

*P<0.05 as a compared to control

DISCUSSION

The haemodynamic changes accompanying haemorrhage include generalised vasoconstriction, particularly in skin, splanchnic area and skeletal muscles. Vasoconstriction reduces perfusion, which causes hypoxia of tissues and the first attack of hypoxia is cell's aerobic respiration and if hypoxia persists, it leads to irreversible injury or cell death (10). Chambers et al (11) reported that during ischaemia there is either excessive production of free radicals or the ability of their disposal by antioxidants is lost. Freeman and crapo (12) defined that oxidative deterioration of polyunsaturated fatty acids (peroxidation of lipids) in biological system can be initiated by free radicals and lipid peroxidation is implicated as a major cause of tissue damage. Peroxidation of unsaturated fatty acids of cellular membranes produces thiobarbituric acid reacting aldehydes and malondialdehyde is one of them. Niehaus (13) reported that MDA is formed during microsomal lipid peroxidation and its level may be considered as an index of lipid peroxidation.

In our study, an increase in plasma MDA level after haemorrhagic shock and after transfusion of blood in all the four groups (Tables I & II) was observed. This rise in level of MDA was statistically significant ($P<0.05$) in group II (which was subjected to most severe shock both in terms of magnitude of hypotension and duration of shock) only after 1 and 2 hrs of blood transfusion. Significant fall in haematocrit value ($P<0.05$) of Group II (Table III) after the maintained shock period confirms the severity of shock. Prasad et al also (14) reported increase in plasma MDA in haemorrhagic shock (50 mm Hg) of 2 hrs duration and after 2 hrs of reperfusion but the results were not significant statistically. The results suggest that lipid peroxidation occurs more with ischaemic reperfusion than the ischaemic injury. Moreover the severity of shock is directly related to the increase in level of plasma MDA. Similarly Gaudel and Du Velleroy (15) reported that concentration of plasma MDA was parallel quantitatively with the functional damage occuring after post ischaemic reperfusion. This suggests that the plasma MDA level may help in knowing the prognosis of patient having haemorrhagic shock. Rhee et al (16) also reported increase in survival rate in rats subjected to haemorrhagic shock with prior administration of antioxidants but co-relation of survival rate with plasma MDA was not done.

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