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Original Article

Protective role of ascorbic acid in hemorrhage-induced cardiovascular depression

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Abstract

Haemorrhagic shock is associated with hyperproduction of free radicals. Hence ascorbic acid (AA), a free radical scavenger, may be of benefit in such cases. We investigated in Wistar rats the role of free radicals in causing cardiovascular depression and the protective role of ascorbic acid, if any, in acute haemorrhage. The rats were catheterized for recording hemodynamic parameters. Acute haemorrhage was induced through phlebotomy followed by resuscitation. The test group received AA for 3 days before inducing haemorrhage and was resuscitated with AA and Ringer's lactate. The controls received only ringer lactate. Malondialdehyde was estimated to assess free-radical production. All rats showed significant fall in cardiovascular parameters with simultaneous increase in MDA level. While all rats showed significant improvement following resuscitation, the recovery was greater in the test group. The MDA levels decreased significantly in the ascorbic acid treated group. Our results demonstrate the involvement of free radicals in hemorrhage-induced cardiovascular depression and the cardio-protective effect of ascorbic acid.

Introduction

Hemorrhagic shock, which is commonly encountered in clinical practice, has been extensively studied experimentally. Acute hemorrhage leads to myocardial ischemia and injury, cardiac depression and hypotension. Myocardial ischemia causes free radical generation by increasing the activity of xanthine and xanthine oxidase which, in the presence of tissue oxygen, produce oxygen free radicals (1). Loss of superoxide dismutase and glutathione peroxidase during ischemia also leads to increase in free radicals (2, 3). Increased free radicals may decrease cardiac performance by decreasing calcium transport and ATPase activity (4). Pretreatment of animals with superoxide dismutase and catalase has been shown to attenuate the toxic effects of acute hemorrhage (2). Oxygen free radicals (OFRs) are involved in acute hemorrhage-induced hemodynamic changes and hence, the use of free radical scavengers might have a protective or therapeutic role in acute hemorrhage.

Ascorbic acid or vitamin C is a redox catalyst found in both animals and plants. It reduces and neutralizes reactive oxygen species such as hydrogen peroxide (5, 6). Besides its direct antioxidant effects, ascorbic acid is particularly important in stress resistance in plants as it is also a substrate for the redox enzyme

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ascorbate peroxidase (7). Hence, ascorbic acid is considered to be a free radical scavenger or an anti-oxidant. In this study, we investigated the role of ascorbic acid as a free radical scavenger and further, its protective and therapeutic role in hemorrhage.

Materials & Methods

Experiments were performed in Healthy Wistar albino rats (either sex), weighing between 250-300 g after taking approval from the Institutional Ethics Committee. The rats were housed in polyethylene cages in groups of four per cage and kept at 25±2°C with a 12-h light/dark cycle. Rats were supplied with water and food *ad libitum*. Ascorbic acid was fed per orally to the rats in the test group. On the day of experiment, rats were anesthetized with urethane dissolved in distilled water and injected intraperitoneally (i.p.) at a dose of 1 gm/kg body weight.

A polyethylene catheter was placed in the femoral artery for recording arterial blood pressure, both systolic blood pressure (SBP) and diastolic blood pressure (DBP) using a pressure transducer (Stathem P23D) and for collecting blood samples. The right femoral vein was cannulated for intravenous infusion. A catheter was placed in left ventricle through right carotid artery for recording *left ventricular pressure* (LVP) using a pressure transducer (Statham p23Db) with Power lab data acquisition system 4/25 (AD Instrument). For myocardial contractility, LV dp/dt max was recorded online by differentiating LVP using Power lab software. Malondialdehyde estimation was done by reaction with Thio Barbituric acid (TBA) (8).

Experiments were performed in 2 groups of animals: In the control group, rats were treated only with Ringer's lactate (RL) infusion (54 ml/kg) over 60 minutes after inducing hemorrhage. In the test group, rats were pre-treated with AA (50 mg/kg/day) for 3 days prior to inducing hemorrhage and also received intravenous infusion of ascorbic acid (50 mg/kg) in RL following hemorrhage.

In both the groups, blood was withdrawn in steps of 10% of the estimated blood volume (7% of body weight) each time, at 10 minutes interval till 40% of estimated blood volume was withdrawn. MDA estimation & continuous measurements of hemodynamic parameters were done. After hemorrhage, resuscitation was done accordingly in both the groups and all measurement repeated.

Graph Pad Prism was used for statistical analysis. Paired t-test was used for comparison within the group. Inter group comparison was done using one way analysis of variance (ANOVA). A p value of <0.05 was used as the level of significance in all statistical tests.

Results

There was a significant fall in all hemodynamic

TABLE I: Change (%) in various hemodynamic parameters after 15, 30 and 60 minutes of resuscitation following 40% blood loss in rats in the control and test groups. P values signify significant changes (improvement) in the test group in comparison to the control group.

Hemodynamic parameters	*Group*	*Percentage change (40% hemorrhage & after 15 min of infusion)*	*Percentage change (40% hemorrhage & after 30 min of infusion)*	*Percentage change (40% hemorrhage & post 30 min of infusion)*
SBP	Control (RL infusion)	8.93±1.42	47.89±19.17	66.84±8.97
	Test (RL+AA infusion)	70.42±29.41	77.73±28.0	92.25±25.25
		p<0.0001	p=0.0027	p=0.0037
DBP	Control	23.27±12.53	42.43±16.47	57.63±15.07
	Test	48.44±25.41	65.78±14.64	83.39±11.97
		p=0.0058	p=0.0018	p=0.0004
LVP	Control	16.89±8.71	30.16±13.76	43.83±9.33
	Test	76.2±16.01	86.9±11.14	97.01±12.78
		p<0.0001	p<0.0001	p<0.0001
LVdp/dtmax	Control	25.36±15.37	35.21±16.04	48.03±9.33
	Test	48.5±26.0	67.45±29.76	89.64±29.44
		p=0.026	p=0.0074	p=0.0013

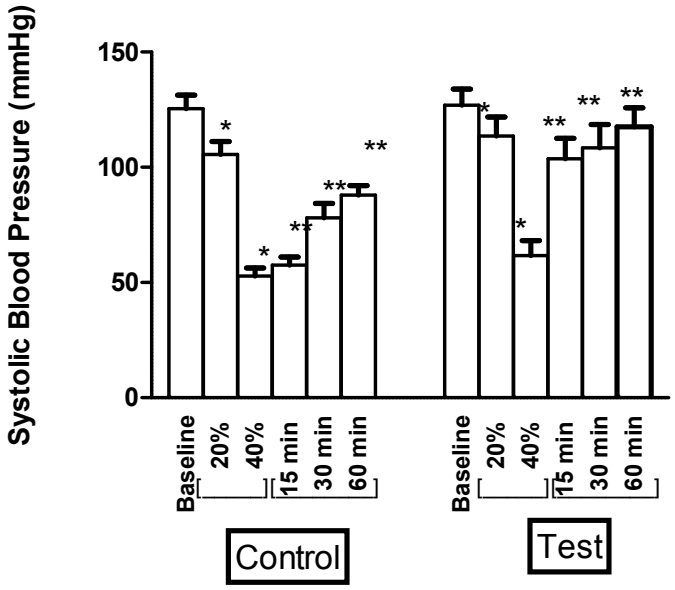

Fig. 1 : SBP (mmHg) in the rats (baseline, after 20% and 40% haemorrhage and after resuscitation by RL and RL+AA infusion) in the control and test groups respectively. All values are expressed as Mean ± SD. '*' shows statistical significant differences between post haemorrhage and baseline values ($p<0.05$). Statistical significant differences between the post treatment values compared to the 40% haemorrhage values is shown by '**' ($p<0.05$).

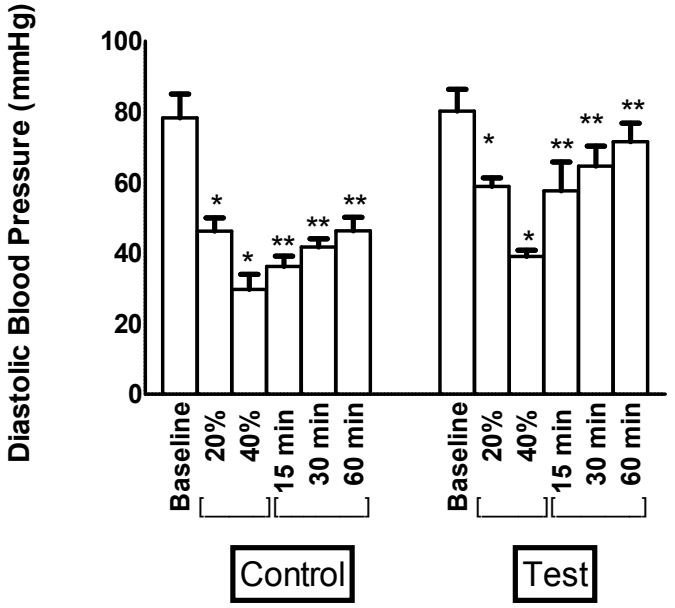

Fig. 2 : DBP (mmHg) in the rats (baseline, after 20% and 40% haemorrhage and after resuscitation by RL and RL+AA infusion) in the control and test groups respectively. All values are expressed as Mean±SD. '*' shows statistical significant differences between post haemorrhage and baseline values ($p<0.05$). Statistical significant differences between the post treatment values compared to the 40% haemorrhage values is shown by '**' ($p<0.05$).

parameters viz. SBP, DBP, LVP, LVdp/dt max on induction of hemorrhage. Following resuscitation, gradual improvement in all parameters was observed in both the groups. Changes in SBP, DBP, LVP, LVdp/dt max have been depicted graphically in Figs. 1, 2, 3 and 4 respectively. These figures show that in the test group, after infusion of RL+AA following hemorrhage, the improvement was almost complete, cardiovascular parameters reverting back to almost normal values (>90% of post hemorrhage value).

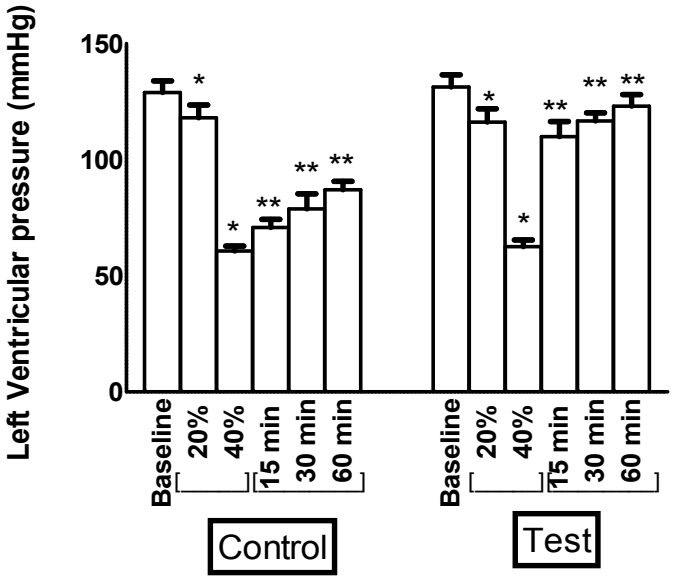

Fig. 3 : LVP (mmHg) in the rats (baseline, after 20% and 40% haemorrhage and after resuscitation by RL and RL+AA infusion) in the control and test groups respectively. All values are expressed as Mean±SD. '*' shows statistical significant differences between post haemorrhage and baseline values ($p<0.05$). Statistical significant differences between the post treatment values compared to the 40% haemorrhage values is shown by '**' ($p<0.05$).

The percentage improvement in the hemodynamic parameters in both the groups is given in Table No. I. There was a significant difference when the post resuscitation mean change in the control group was compared to the mean change in the test group.

The MDA levels at different steps during the experiment are shown in Fig. No. 5. There was a rise in MDA level on inducing hemorrhage in both the groups, indicating that prior administration of AA has no preventive effect against production of free radicals. Though, AA+RL infusion in the test group

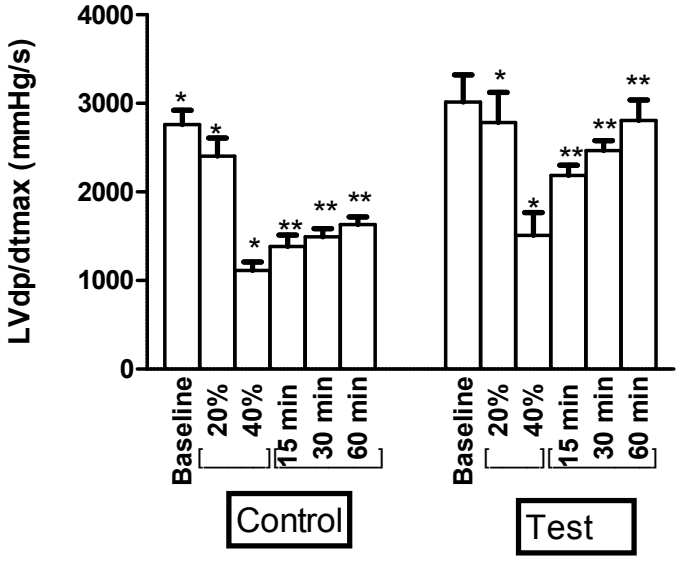

Fig. 4 : LVdp/dtmax (mmHg/s) in the rats (baseline, after 20% and 40% haemorrhage and after resuscitation by RL and RL+AA infusion) in the control and test groups respectively. All values are expressed as Mean±SD. '*' shows statistical significant differences between post haemorrhage and baseline values ($p<0.05$). Statistical significant differences between the post treatment values compared to the 40% haemorrhage values is shown by '**' ($p<0.05$).

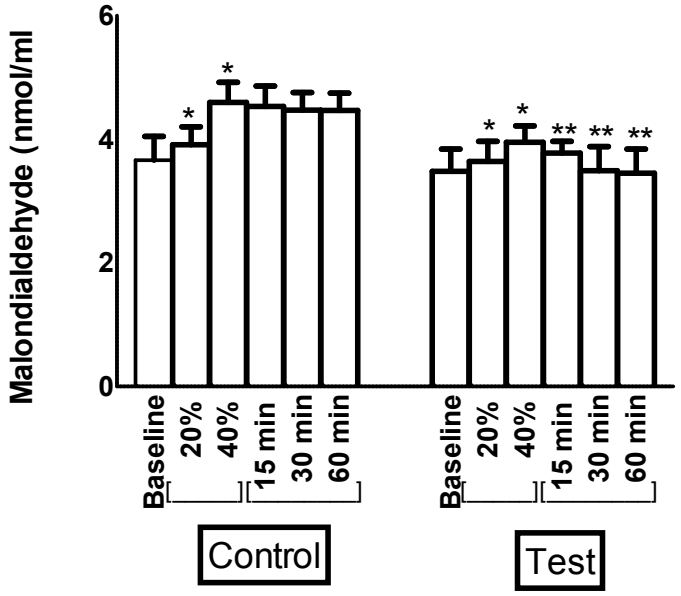

Fig. 5 : Malondialdehyde (nmol/ml) levels in the rats (baseline, after 20% and 40% haemorrhage and after resuscitation with RL and RL + AA in the control and test groups respectively. All values are expressed as Mean±SD. '*' shows statistical significant differences between post haemorrhage and baseline values ($p<0.05$). Statistical significant differences between the post treatment values compared to the 40% haemorrhage values is shown by '**' ($p<0.05$).

resulted in significant fall in MDA levels, whereas the MDA levels remain elevated after infusion of RL alone.

Discussion

Hemorrhage depresses overall cardiac function and contractility as observed in our study and also reported by others (911). One of the important factors leading to depressed cardiovascular functions during hemorrhage is the genesis of free radicals, documented by many and confirmed in our study (2, 4, 912). In our study, free radicals were increased as indicated by elevated levels of malondialdehyde, a stable product of lipid peroxidation and corroborated by using ascorbic acid, a free radical scavenger, to protect against the deleterious effect of blood loss.

Free radicals are shown to depress Ca^{++} transport and Ca^{++} ATPase of sarcoplasmic reticulum, leading to a decrease in myocardial contractility as well as the rate of relaxation (13). Administration of enzymatic free radicals like superoxide dismutase and catalase has shown protection against acute hemorrhage (2, 11). Similarly, in this study, we have studied the role of ascorbic acid in overcoming the deleterious effect of acute blood loss. In our study, we tried to investigate prophylactic as well as the therapeutic role of AA in acute hemorrhage. We observed that pretreatment with ascorbic acid did not have any beneficial effect, as the extent of cardiovascular depression following hemorrhage was comparable in both the groups. However the recovery was almost complete (similar to the baseline values) in AA treated group than in the control group. MDA levels were found to be elevated during hemorrhage in both the groups.

Contrary to our findings, Daughters K et al have shown AA to be not of much benefit in hemorrhagic shock, rather they proved that vitamin K improves survival in such cases (14). Similarly, Minor T and co-workers found no evidence for a protective effect of AA on free radical generation and liver injury after hemorrhagic shock in rats (15). In this study, ascorbic acid was administered prior to induction of hemorrhage. On the other hand, lyophilized plasma with ascorbic acid has documented decreased

inflammation in hemorrhagic shock by decreasing IL-6 expression (16, 17). Increased incidence of hemorrhage in seen in surgical patients with vitamin C deficiency but normal coagulation studies, this is reversed by vitamin C replacement (18). Similarly, plasma vitamin C levels decreased and co-related to brain damage in patients with intracranial hemorrhage (19).

Similar to our findings in hemorrhage, benefits of free radical scavengers like vitamin C and E has also been demonstrated in ischemia reperfusion injury, anaphylactic shock and in thermal injuries (12, 20 22).

We found that ascorbic acid was beneficial in hemorrhagic rat model and suggest the involvement of free radicals in the pathogenesis of cardiovascular dysfunction during hemorrhage. We conclude that adding ascorbic acid in the resuscitation fluid may benefit the patients with hemorrhagic shock and such practice is recommended after clinical trials.

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