EFFECTS OF REDUCED GLUTATHIONE ON EXPERIMENTAL HEMORRHAGIC SHOCK

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ABSTRACT

Effects of reduced glutathione (GSH) were evaluated in animals subjected to hemorrhagic shock. In this study using rabbits, three phases were shown in the pattern of bleeding, i.e., bleeding phase, quiescent phase and reflux phase with resultant animals' death. We observed marked disturbance in the levels of histamine during the course of hemorrhage. Administration of GSH abolished reflux phase, indicating well maintained vascular tone, and an exaggerated release of plasma histamine was significantly suppressed. In rats, microcirculatory disorders as well as the degranulation rate of mast cells in the mesentery were significantly prevented by the administration of GSH. Thus GSH seems to exert salutary effects on hemorrhagic shock primarily by the maintenance of vascular tone and suppression of the release of chemical mediators.

INTRODUCTION

Since the excellent work by Dale,¹ an appreciable advance has been made in the study of shock in terms of histamine release. Unfortunately, however, the role of histamine is still incoherent. Considering the observation that microcirculatory system varies moment-to-moment during the course of shock, an investigation of sequential changes in plasma histamine is necessary to solve the lying problem in this disorder.

Recently, reduced glutathione (GSH)²⁻⁵ as well as cysteine⁶ has been reported to have salutary effects on various forms of shock.

The present study was designed to elucidate the relationship between the

microcirculatory derangement and the mode of action of plasma histamine along with some biochemical aspects of GSH in hemorrhagic shock.

MATERIALS AND METHODS

The study is composed of the following:

(I) Experiments with rabbits

Male albino rabbits (2.5–3.0 kg) were used and were divided into the following. Each group consisted of 5 animals. (1) control (hemorrhage alone). (2) GSH was given in a dose of 300 mg/kg iv 30 min prior to hemorrhage. (3) 1,000 mg/kg of body weight of GSH was given ip 180 min prior to hemorrhage. (4) received GSH (100 mg/kg) iv every 15 min during hemorrhage.

Under intraperitoneal anesthesia with sodium pentobarbital (30 mg/kg), polyethylene catheters were inserted in the left femoral artery and the left femoral vein. The femoral artery catheter was connected to the reservoir, which was fixed at 30 cm in height and the femoral vein catheter was used for the drawing of blood samples and for infusion. GSH (Tathion, Yamanouchi Pharmaceutical Co. Tokyo) was dissolved in pyrogen-free solution (300 mg/ml).

An improved method of fluorometric assay, reported previously by the authors,⁷ was used for detecting plasma histamine. Six ml of heparinized blood samples were taken from the femoral artery before and every 15 to 30 min of bleeding for 90 min.

(II) Experiments with rats

Wistar strain male albino rats weighing 250–300 g were used for the experiment. Animal groups and procedures used in these groups were as follows. (1) control (bleeding alone). (2) hemorrhage plus intravenous administration of 1 ml of 0.9% NaCl. (3) GSH was injected in a dose of 100 mg/kg, dissolved in 1 ml of solution, iv in the course of bleeding. (4) 1,000 mg/kg of GSH, dissolved in 1 ml of solution, was given iv during the course of hemorrhage.

Rats were anesthetised with sodium pentobarbital (30 mg/kg) given ip. Polyethylene catheter was inserted in the abdominal aorta and was connected to the reservoir, which was fixed at 15 cm in height. The left juglar vein was cannulated for infusion. Changes in the mesenteric microcirculation were carried out, using closed circuit high-resolution television-microscopy, in all these groups. Mesentery mast cells were stained with 0.1% toluidine blue and the number of degranulation was counted.

RESULTS

(I) Sequential changes in the blood volume obtained from the control group of rabbits are given in Fig. 1 where three phases are demonstrated, i.e., the bleeding phase (initial bleeding by 30 min), the quiescent phase (the next 30 min) and the reflux phase (the subsequent period to the animals' death). Approximately 35 ml of blood was withdrawn at 5 min after initiating hemorrhage. This rapid bleeding lasted for 30 min and the volume obtained at 30 min after bleeding was approximately 55 ml, which indicated 1.6 fold of the initial 5 min's value. The next 30 min was the quiescent phase where slight increase was found in the blood volume, and was followed by the reflux phase. The last phase showed reflux with resultant 60% of blood return within 30 min.

Figure 2 illustrates the changes in the bleeding rate examined in four groups of rabbits. The bleeding rate indicates the ratio compared to the value at 5 min of bleeding. No marked difference was seen in the second group compared to the control in a pattern of bleeding. In the third group, the reflux of

Fig. 1 Sequential changes in the bleeding volume in the control of rabbit ($m \pm SD$).

Fig. 2 Sequential changes in the bleeding rate for five animals in each group of rabbits. ●—● control, ●—● administered 300 mg/kg of GSH iv 30 min prior to hemorrhage, ●—● 1,000 mg/kg of GSH ip 180 min prior to hemorrhage, ●---● 100 mg/kg of GSH is every 15 min after bleeding.

the blood was gradual and its rate at 90 min revealed to 25% of the bleeding volume. On the other hand, on administering 100 mg/kg of GSH iv to the rabbits every 15 min after hemorrhage, little reflux was seen.

Figure 3 depicts sequential changes in the levels of plasma histamine in the control. Average (ISD) value of plasma histamine in controls was 43 ± 3.8 $\mu\text{g/L}$. During 30 min of hemorrhage, the levels increased rapidly in a fashion similar to the blood volume and attained a maximum at 90 min after bleeding, giving 62.4 ± 4.4 $\mu\text{g/L}$ ($m \pm \text{SD}$).

Sequential changes in the levels of plasma histamine in these group are presented in Fig. 4. Neither suppression nor significant difference was shown in the mode of action of the amine in a group treated beforehand with GSH (300 mg/kg) compared to that of controls.

In contrast, plasma histamine level was significantly suppressed in the animals which were either pretreated with 1,000 mg/kg GSH or GSH at 15 min intervals during the course of hemorrhage, i.e., under both conditions the values obtained at 15 min, 30 min and 60 min revealed $p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively.

(II) In rats, consecutive bleeding developed by 30 min where the volume attained a peak and showed approximately 20 ml/kg of body weight. Investigation of the rat mesenteric microcirculation yielded clearly the relationship be-

Fig. 3 Sequential changes in the levels of plasma histamine in the control group of rabbit ($m \pm SD$).

Fig. 4 Changes in the levels of plasma histamine in hemorrhagic shock in rabbits expressed as means $\pm$ SD. control, 300 mg/kg of GSH iv 30 min prior to hemorrhage, 1,000 mg/kg of GSH ip 180 min prior to hemorrhage, 100 mg/kg of GSH iv every 15 min after bleeding.

tween the changes of plasma histamine and vascular system during the course of hemorrhagic shock. In the initial stage of hemorrhage (bleeding phase), striking vasoconstriction occurred in the arterioles, which was followed by stasis, sludge phenomena, white thrombus formation, increased permeability and diapedesis in the venules. In the subsequent phase, dilatation of venules was prominent with concomitant increased permeability and reflux of the blood in the microcirculatory system.

Salutary effects of GSH on the microcirculatory system was demonstrated by the initial administration of 1,000 mg/kg of body weight, indicating improvement of the above-mentioned distress. This led to further bleeding with resultant quiescent phase in which vascular stasis as well as sludged blood was marked. However, it should be emphasized that treatment with same procedure at this stage also dramatically reversed to seemingly normal stream.

Figure 5 illustrates the degranulation rate of mesentery mast cells obtained at 30 min of bleeding. Degranulation rate was $94.3 \pm 5.4\%$ ($m \pm SD$) in the controls together with in the group hemorrhage plus infusion of 1.0 ml of 0.9% NaCl ($88.9 \pm 10.5\%$, $m \pm SD$), showing small amount of NaCl had no suppressive effect on degranulation of mesentery mast cells. However, degranulation was prevented in the group received 100 mg/kg of body weight of GSH ($84.4 \pm 1.9\%$, $m \pm SD$), demonstrating to permit statistical significance ($p < 0.05$). Further-

Fig. 5 Degranulation rate of mesenteric mast cells in hemorrhagic shock in rats. ▨ control (hemorrhage alone), ▤ hemorrhage plus 1 ml of 0.9% NaCl iv, ▧ hemorrhage plus 100 mg/kg of GSH iv, ▩ hemorrhage plus 1,000 mg/kg of GSH iv.

more, if treated with GSH (1,000 mg/kg) during hemorrhage, the rate decreased extremely by up to $3.8 \pm 3.0\%$ ($m \pm SD$), which was statistically highly significant ($p < 0.001$).

DISCUSSION

Beneficial effects of GSH and related agents have been demonstrated on various forms of shock. Van Caneghem⁸ suggested that the mechanism of thiol substances was to inhibit the cathepsic activity and stabilize the lysosomes. Jokay *et al.*⁹ reported that SH-compounds inhibited *in vitro* the endotoxin-induced platelet agglutination, histamine and serotonin release. Be this as it may, microcirculatory derangement is a prominent feature of shock per se, and this may initiate a more or less profound perturbation of the entire integrated environment.

The first phase of the hemorrhage shown in this study is a stage in which vasoconstriction is enhanced by peripheral resistance vessels owing to hemorrhage. However, it results in a damage of vascular endothelial cells concomitant with a release of vasoactive substances, including histamine. The second phase implied steady stage between constriction and dilatation of the vascular system, and if prolonged, irreversible stasis will ensue, i.e., progressive and irreversible vasodilatation (reflux phase). The last phase developed some 60 min after bleeding, which inferred the beginning of atonic state and the reflux rate reached up to 90% consequent upon animals' death.

The levels of plasma histamine have been thought to have intimate relations with the degranulation of mast cells.¹⁰ Indeed, hemorrhagic shock was high in the present study. A vasoactive substance such as histamine, which readily undergoes considerable configurational changes in many circumstances, would therefore be expected to induce a microcirculatory distress in a significant proportion of individuals. Thus, it is not far-fetched to consider that without restoration of this system, no improvement may be anticipated in shock, and the role of histamine should never be ignored.

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