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Ranitidine and oxygen derived free radical scavengers in haemorrhagic shock induced gastric lesions

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SUMMARY **The role of oxygen derived free radicals in gastric lesions induced by haemorrhagic shock and the protective effect of oxygen radical scavengers, allopurinol and ranitidine, were investigated. Forty five rabbits underwent haemorrhagic shock for 30 minutes and reinfusion of shed blood. They were killed 30 minutes later. The animals were divided in five groups: A (n=10): Control, B (n=10): intravenous ranitidine pretreatment, C (n=10): oral allopurinol, 24 and 2 h before surgery, D (n=10): intravenous pretreatment with superoxide Dismutase plus catalase, E (n=5): 60 minute haemorrhagic shock without reinfusion and treatment. Erosions and/or petechiae in all animals in Group A were observed. Three animals in group B and C and 2 in group D ($p<0.005$, $p<0.001$) had gastric lesions. The lesions in the pretreatment groups were significantly smaller than in controls. Oxygen radicals plus HCl play an important role in shock induced gastric lesions. Oxygen radical antagonists show a significant protective role.**

Clinical and experimental studies have shown that haemorrhagic shock often is followed by rapid development of gastric mucosal lesions.[1-3] Gastric blood flow is considerably reduced during haemorrhagic shock. In the fundic mucosa the reduction of blood flow is more extensive than in the antral mucosa.[4] A number of hypotheses have been proposed to explain the mechanism of ischaemia induced gastric lesions, but the pathogenesis remains uncertain.[2-7]

Recent evidence suggests that oxygen derived free radicals may be abundantly produced in post-ischaemic tissues, accounting for at least part of the damage.[8] Furthermore, it has been reported that superoxide radicals ($\cdot O_2^-$) play an important role in the formation of gastric lesions produced by ischaemia plus HCl.[9] Hydroxyl radical ($OH^\cdot$) appears to be the major oxygen radical contributing to ischaemic damage of gastric musoca.[10]

The present experiments were designed to study the role of oxygen derived free radicals in haemorrhagic shock induced gastric lesions and the protective effect of oxygen radical scavengers, allopurinol (a xanthine oxidase inhibitor) and ranitidine (an H_2 receptor antagonist), in the rabbit stomach.

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Methods

ANIMALS

Forty five female white rabbits weighing 1200–2000 g, were fasted for 24 hours before the experiments but were allowed water *ad libitum*. Each animal was anaesthetised with ketamine (50 mg/kg body weight) plus fentanyl (0·025 mg/kg) and diazepam (5 mg/kg). External jugular vein was catheterised for the administration of the drugs. Carotid artery was canulated using a 22 gauge catheter. Another 22 gauge catheter was inserted into the femoral artery in order to withdraw and reinfuse blood. Laparotomy was undertaken and the gastro-esophageal junction and duodenum (2 cm distal to the pylorus) were ligated. A small gastrotomy was carried out at the greater curvature of the rumen and a tube was passed through the gastrotomy for gastric

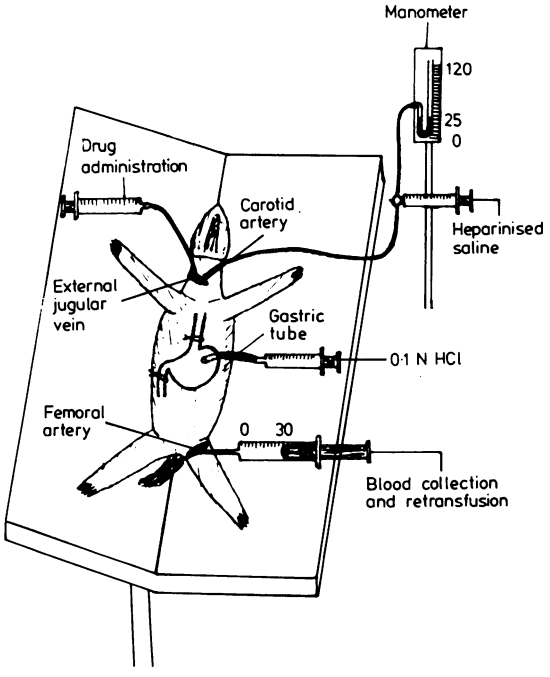

Figure *Schematic representation of the experimental model.*

lumen lavage (Figure). One millilitre 0·1 N HCl/100 g body weight[9] was instilled into the stomach through the gastric tube. The tube was then removed and the gastrotomy was sutured with Vicryl No 4/0. Blood was withdrawn from the femoral artery into a syringe containing 1·5 ml heparinised saline (100 U heparin/ml) until the mean blood pressure was reduced to 20–30 mmHg. The blood pressure was maintained at this level for 30 min, the shed blood was then reinfused. Thirty minutes later the animal was killed by decapitation and the stomach was removed. The pH of the gastric juice was determined at the end of the shock period and the end of the experiment. The stomach was opened along the greater curvative and the gastric mucosal lesions were identified using a dissecting microscope with ×25 magnification. The area of the gastric lesions was measured in mm^2 as previously described.[9] The stomach was then fixed in 7% formalin.

Five groups of rabbits have been included in this study. The drug pretreatment regimens were as follows: group A (n=10): no pretreatment (control group); group B (n=10) ranitidine 1 mg/kg intravenously through the jugular vein as bolus 15 min before the removal of blood; group C (n=10): allopurinol 50 mg/kg orally through an oragastric tube, 24 and two hours before surgery; group D (n=10): superoxide dismutase plus catalase, each 15 000 U/kg iv through the jugular vein as bolus immediately before induction of shock; group E (n=5): no pretreatment. Sixty minute haemorrhagic shock without reinfusion of the blood.

Table 1 *Mean blood pressure (mean (SD)) before and during the shock and retransfusion periods*

		Blood pressure (mmHg)		
Group	*n*	*Prebleeding*	*Postbleeding*	*Postreinfusion*
A	10	118·1 (17·3)	24·9 (1·8)	117·5 (15·5)
B	10	114·6 (22·8)	25·0 (1·6)	110·0 (10·5)
C	10	107·4 (19·4)	24·9 (1·7)	109·0 (10·1)
D	10	117·0 (9·2)	25·0 (1·6)	116·0 (6·5)
E	5	129·2 (14·3)	24·8 (1·9)	—

STATISTICAL ANALYSIS

Student's *t*, Hodges-Lehman (non-parametic procedure) and χ^2 tests were used to analyse the data.

Results

The removal of about 19 ml/kg body weight of blood caused a profound decrease in the arterial pressure of the animals. The pressure almost returned to baseline values after restoration of the normal blood volume (Table 1).

The gastric juice pH was about 1·0 in all groups after the instillation of 0·1 N HCl. No change of pH was present after a 30 minute haemorrhagic shock period. At the end of the experiment, 30 minutes after the reinfusion of shed blood, a statistically significant increase of gastric juice pH of group B *v* group A was noted (Table 2).

In the control group (group A), all animals showed gastric mucosal erosions and/or petechiae. Statistically significant decrease of the incidence and severity of gastric mucosal lesions was presented in all pretreatment groups (Table 3). In the animals of group A the formation of mucosal lesions occurred

Table 2 *Values of gastric juice pH (mean (SD))*

	Gastric juice pH		
Group	*Prebleeding*	*At the end of the shock*	*At the end of the experiment*
A	1·06 (0·06)	1·07 (0·05)	1·50 (0·25)
B	1·05 (0·07)	1·05 (0·06)	2·43 (0·65)*
C	1·03 (0·06)	1·06 (0·05)	1·93 (0·80)
D	1·06 (0·05)	1·08 (0·06)	1·40 (0·34)
E	1·02 (0·04)	—	1·04 (0·05)

*$p<0·01$ *v* group A (control) at the same period (*t* test).

Table 3 *Incidence and total lesion area after haemorrhagic shock and retransfusion-induced gastric lesion*

Group	n	Rabbits with gastric lesions (n)	Total lesion area in mm^2 (mean (SD))
A	10	10	86·6 (54·4)
B	10	3*	10·1 (28·2)§
C	10	3†	20·6 (56·4)‖
D	10	2‡	7·5 (22·0)¶
E	5	0	—

*$p<0·005$ *v* group A using χ^2 test; †$p<0·005$ *v* group A using χ^2 test; ‡$p<0·001$ *v* group A using χ^2 test; §$p<0·0005$, $\chi^2=12·1$ *v* group A using Hodges-Lehman test; ‖$p<0·0026$, $\chi^2=9·07$ *v* group A using Hodges-Lehman test; ¶$p<0·00044$, $\chi^2=12·4$ *v* group A using Hodges-Lehman test.

mainly in the corpus. The total area of the gastric lesions was 86·6 (54·4) mm^2. Values are expressed as mean (SD). Only in one animal lesions were found in the duodenum (30 mm^2). In another animal lesions were found in the antrum (45 mm^2). Significant decrease of the total lesion area was presented in the pretreatment groups (Table 3).

The animals in group E died 40–50 minutes after the blood withdrawal. No change of gastric juice pH was found. None of the animals developed gastric mucosal lesions.

The presence of mucosal lesions was confirmed by histological examination. The microscopic examination of the erosions revealed a loss of the superficial layers (lining epithelium and gastric pits) and a destruction of the normal architecture of the upper part of the gastric mucosa. Intact cells of the gastric glands could still be observed but the normal parallel arrangement of the cellular elements of the glands could not be detected at the upper part of the mucosa. Marked congestion of the mucosa and small subepithelial haemorrhagic areas were frequently found at or near the erosions.

Discussion

In the present study a 30 minute period of haemorrhagic shock followed by a 30 minute period of reinfusion of the shed blood led to the appearance of mucosal lesions mainly in the corpus of the stomach. This topographical predilection could be explained on the basis of a higher susceptibility of the corpus to the haemorrhagic shock. This may be because of the greater degree of circulatory disturbance and the higher vulnerability to ischaemia of this region.[11] In anaesthetised dogs during the shock, the blood flow to fundic mucosa decreases more extensively than the flow to antral mucosa.[4]

Gastric mucosal permeability to the back diffusion of hydrogen ion is increased by many chemical agents.[6 12] A short period of haemorrhagic shock (40 mmHg mean arterial pressure for 15 minutes) had a disruptive effect on the gastric mucosal barrier to the hydrogen ion. This effect is similar to that produced by certain chemical agents.[6] Gastric acid is an indispensable factor in the pathogenesis of gastric lesion formation. Acid induces the formation and extension of gastric mucosal injury in circumstances in which the gastric mucosa is initially damaged by other factors.[9] In our experiments, pretreatment with ranitidine, an H_2 receptor antagonist, significantly decreased the gastric mucosal lesions. At the end of the experiment there were some increase in the level of pH in the reinfused groups, except group B, but the difference was not statistically significant. In ranitidine group (group B) a statistically significant increase of pH was noted. It is known that 0·1 N HCl alone does not produce mucosal injury.[9]

Prolonged ischaemia followed by reperfusion produces morphological alterations of the tissues including endothelial cell swelling, interstitial oedema and necrosis.[13 14] Haemorrhagic shock may be viewed as a 'whole body ischemia'.[8] All the tissues may be insufficiently perfused. During the shock, ATP concentrations are drastically reduced in many organs[15] resulting in raised hypoxanthine concentrations in plasma.[16 17] Reconstitution of the intravascular volume fully restores microvascular perfusion and tissue oxygenation, setting the stage for a reperfusion injury. In the renewed presence of molecular oxygen, xanthine oxidase reacts with hypoxanthine to produce the cytotoxic superoxide radical ($\cdot O_2^-$):

$$\text{Hypoxanthine} + O_2 \xrightarrow[\text{oxidase}]{\text{xanthine}} \text{Xanthine} + \cdot O_2^-$$

Superoxide dismutase efficiently and specifically scavenge the superoxide radical by catalysing its dismutation to hydrogen peroxide and oxygen:[18]

$$\cdot O_2^- + \cdot O_2^- + 2H^+ \xrightarrow[\text{dismutase}]{\text{superoxide}} H_2O_2 + O_2$$

Hydrogen peroxide which is a relatively non-toxic substance can be scavenged by catalase to form water and molecular oxygen:[18]

$$H_2O_2 + H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Evidence to support this hypothesis is provided by the observation that allopurinol substantially increased the survival rate of dogs subjected to haemorrhagic shock.[16 19] Recent studies suggest that oxygen derived free radicals play an important role in the formation of gastric lesions produced by ischemia plus HCl.[9 10] Particularly $\cdot O_2^-$ in the rat stomach or $OH^\cdot$ in the cat stomach[10] appear to be the major oxygen radicals contributing to ischaemic damage.

In the present study superoxide dismutase, a $\cdot O_2^-$ scavenger, plus catalase, an H_2O_2 scavenger, significantly decreased gastric lesion formation induced by haemorrhagic ischaemia. Allopurinol, a xanthine oxidase inhibitor, significantly decreased gastric lesion formation as well. These drugs, allopurinol and free radical scavengers, had the same protective effect on the gastric mucosa as ranitidine, an H_2 receptor antagonist. The H_2 receptor antagonists are well known and effective drugs for prevention and treatment of stress ulcers.[20 21]

In conclusion, the findings of the present study suggest that oxygen derived free radicals play an important pathogenetic role in the haemorrhagic shock induced gastric mucosal lesions in the rabbit stomach. Oxygen radicals and HCl have about the same injurious effect in the gastric mucosa, while both are needed to produce the mucosal injury. Antagonists of oxygen radicals or HCl play a significant protective role against the haemorrhagic shock induced stress ulcers.

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