Effects of Cimetidine, a Histamine Type 2 Receptor Antagonist, on Microcirculatory Disturbance of the Stomach Induced by Full-Thickness Burn Injuries as a Model for Surgical Stress in Rats

Hideki Ishikawa, MD, PhD,¹ Masashi Yoshida, MD, PhD,²
Go Wakabayashi, MD, PhD,² Yoshihide Otani, MD, PhD,²
Motohide Shimazu, MD, PhD,² Tetsuro Kubota, MD, PhD,²
Koichiro Kumai, MD, PhD,² Naoki Aikawa, MD, PhD,¹ and
Masaki Kitajima, MD, PhD²

Departments of ¹Emergency and Critical Care Medicine and ²Surgery, Keio University School of Medicine, Tokyo, Japan

ABSTRACT

Background: We have reported the preventive effect of cimetidine, a histamine type 2 receptor antagonist, on decreased gastric mucosal blood flow induced by burn injury, a model of surgical stress.

Objective: The present study was performed to assess the effects of cimetidine on gastric microcirculatory disturbance induced by surgical stress.

Methods: Twelve male Wistar rats were anesthetized and a 30% full-thickness dorsal scald burn was inflicted. The total lengths of gastric erosions were measured using stereoscopic microscopy. Microvascular images in the basal region of the gastric mucosa were observed using an intravital microscope, and the diameters of venules and collecting venules were measured. Rolling leukocytes inside and along the venules, an indication of endothelial damage to the microcirculation, were observed. The rats were assigned to the cimetidine group or the control group (both, n = 6). Cimetidine 100 mg/kg was administered 30 minutes before and 2.5 hours after infliction of the burn injury. Animals in the control group were given only isotonic saline. Values are expressed as mean (SD).

Results: Contraction of venules was easily observed in all rats in the control group. However, venular contraction was rarely observed in the cimetidine group. The total length of gastric erosions was significantly decreased in the ci-

This article was presented in part in the free paper session at International Surgical Week '97, Acapulco, Mexico, August 24–30, 1997 (Session 133.07, Abstract 711).

Accepted for publication May 27, 2004.

Reproduction in whole or part is not permitted.

doi:10.1016/j.curtheres.2004.07.001
0011-393X/04/\$19.00

metidine group compared with the control group (0.93 [0.58] μm vs 5.98 [5.18] μm , respectively; $P < 0.05$). The percentages of rolling leukocytes that passed the confluence of a prevenue and a venule were also significantly decreased in the cimetidine group compared with the control group (4.7% [7.3%] vs 22.6% [5.7%]; $P < 0.01$). The diameters of the venules and collecting venules were significantly larger in the cimetidine group (57.3 [6.8] μm and 75.9 [3.6] μm , respectively) than in the control group (30.9 [9.2] μm and 46.8 [8.0] μm , respectively) (both, $P < 0.01$).

Conclusions: The present study suggests that cimetidine may have a protective effect against gastric microcirculatory disturbances induced by burn injury, a model of surgical stress. (*Curr Ther Res Clin Exp.* 2004;65:373–382) Copyright © 2004 Excerpta Medica, Inc.

Key words: histamine type 2 receptor antagonist, cimetidine, thermal injury, gastric microcirculation, gastric mucosal lesion, endothelial damage.

INTRODUCTION

In 1967, Davenport¹ reported the disruption of the gastric mucosal barrier to hydrogen ions as a causative factor in the development of gastric mucosal lesions. This theory is now widely accepted, and impairment of defense mechanisms is said to be important in gastric mucosal lesion formation after surgical stress, including burn injury.² We have also reported that gastric mucosal lesions and gastric microcirculatory disturbances are closely related.^{3,4}

It is thought that histamine type 2 receptor antagonists (H_2RAs) reduce the defense mechanisms of gastric mucosa without surgical stress.⁵ But clinically, H_2RAs are often administered to prevent gastric mucosal lesions after surgery^{6,7} or burn injury.⁸

We have reported that cimetidine, an H_2RA , prevents the reduction of gastric mucosal blood flow after burn injury.⁹ The present study was performed to assess the effects of cimetidine on gastric microcirculatory disturbance induced by surgical stress.

MATERIALS AND METHODS

Animals

The protocol of this study followed the animal experimentation guidelines of Keio University School of Medicine (Tokyo, Japan). Twelve male Wistar rats weighing 220 to 270 g were fasted for 24 hours before the study, but were allowed free access to water. They were anesthetized using ether. A 30% full-thickness dorsal scald burn was inflicted by placing the rats in scalding water for 15 seconds as described previously.¹⁰ The animals showed no pain reaction. Just after scalding (within 30 seconds), 50 mL/kg of isotonic saline was injected

intraperitoneally for water resuscitation, as described previously.^{11,12} All of the following assessments were performed 5 hours after infliction of the burn injury.

Mucosal Erosions and Microvascular Images

The lengths of gastric erosions were measured using stereoscopic microscopy, and the total length of all erosions per rat was calculated. Microvascular images in the basal region of the gastric mucosa¹³ were observed using an intravital microscope (Flovel Company, Ltd., Tokyo, Japan).^{14,15} Partial resection of the serosa of the gastric wall was performed, the stomach was mounted on a plastic stage, and a light rod was inserted through an incision in the forestomach. Microcirculatory images were recorded using a charge-coupled video camera system. Using videotaped images, the diameters of venules were measured at the confluence of a prevenue and a venule. The diameters of collecting venules were also measured. Measurements were performed at 3 collecting venules and at 3 confluences of a prevenue and a venule by an assessor who was blinded to treatment assignment. The means of these measurements were used for analysis.

Leukocyte Adhesion on Microcirculation

Approximately 5 minutes before burn injury was inflicted, 0.1 mL of 15.6 mmol/L 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (Molecular Probes, Inc., Eugene, Oregon) was administered intravenously to mark the leukocytes.¹⁶ Illuminated cells were monitored by fluorescence microscopy using a silicon intensifier target image tube camera (SIT camera, Hamamatsu Photonics KK, Shizuoka, Japan). Leukocytes rolling inside and along the venules in the basal layer of the gastric mucosa, an indication of damage to the microcirculation, were observed on videotape. Leukocytes passing through the confluence of a prevenue and a venule were counted for 3 minutes, and the percentage of rolling leukocytes was calculated. Leukocytes were counted at 3 confluences in each rat, and mean of these 3 counts was used for analysis.

Active Oxygen Species Generated by Leukocytes

The generation of active oxygen species (AOS) by whole blood obtained from the left gastric veins was measured using the chemiluminescence (ChL) method as described previously.^{17,18} Whole blood (0.1 mL) obtained from the left gastric veins was diluted with 0.4 mL of minimum essential medium (Eagle MEM, without phenol red, Nissui Seiyaku, Tokyo, Japan). The diluted whole blood was placed in the ChL analyzer (Berthold, Bad Wildbad, Germany), then stimulated by opsonized zymosan to measure luminol-dependent ChL activities after supplementation with luminol (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan). The number of leukocytes in the whole blood was counted, after which the peak of ChL counts per 2 seconds per leukocyte (counts/2 s per leukocyte) was calculated.

Experimental Groups

The rats were assigned to the cimetidine group or the control group (both, $n = 6$). Cimetidine 100 mg/kg PO was administered by gavage to the rats in the cimetidine group 30 minutes before and 2.5 hours after the burn injury was inflicted. Animals in the control group received only isotonic saline PO, also by gavage.

Statistical Analysis

Statistical analysis was performed using the Mann-Whitney U test. Data are expressed as mean (SD). Statistical significance was set at $P \leq 0.05$. Statistical analysis was performed using Tokei-Kaiseki software version 1.5 (Esumi, Tokyo, Japan).

RESULTS

Total Lengths of Erosions

Total lengths of gastric erosions were 0.93 (0.58) μm in the cimetidine group and 5.98 (5.18) μm in the control group ($P < 0.05$; **Figure 1**).

Microvascular Images

Contraction of venules was easily observed in all rats in the control group (**Figure 2A**). However, venular contraction was rarely observed in the cimetidine group (**Figure 2B**). No significant findings were observed in the arteriolar walls.

Diameters of Venules and Collecting Venules

The diameters of the venules were 57.3 (6.8) μm in the cimetidine group and 30.9 (9.2) μm in the control group ($P < 0.01$; **Figure 3A**). The diameters of the collecting venules were 75.9 (3.6) μm in the cimetidine group and 46.8 (8.0) μm in the control group ($P < 0.01$; **Figure 3B**).

Rolling of Leukocytes

The percentages of rolling leukocytes that passed the confluence of a pre-venule and a venule were 4.7% (7.3%) in the cimetidine group and 22.6% (5.7%) in the control group ($P < 0.01$; **Figure 4**).

Chemiluminescence Activity Generated by Leukocytes from Gastric Veins

Luminol-dependent, zymosan-stimulated ChL values from gastric veins were 2353.1 (977.1) counts/2 s per leukocyte in the cimetidine group and 3358.2 (1727.5) counts/2 s per leukocyte in the control group (**Figure 5**). This difference did not reach statistical significance.

Figure 1. Total lengths of erosions in rats receiving cimetidine or saline 5 hours after infliction of the burn injury. * $P < 0.05$.

Figure 2. Microvascular images 5 hours after the infliction of burn injury. (A) Contraction of venules (*) was easily observed in all rats in the control group. (B) Venular contraction was rarely observed in the cimetidine group.

Figure 3. Diameters of (A) venules and (B) collecting venules in rats receiving cimetidine or saline, 5 hours after the infliction of burn injury. * $P < 0.01$.

DISCUSSION

It is thought that microcirculatory disturbance is an important factor in gastric erosion.^{3,19} In the present study, venular contractions were mild in the cimetidine group. We have previously reported that contraction of venules was easily observed in all untreated rats 5 hours after infliction of a burn injury.⁴ Venular contraction could be observed in the noninjured group in poor-quality preparation. However, even if the preparation was well done, venular contraction was easily observed after 5 hours.^{4,12} In a study by Yoshida et al,²⁰ venular contraction was not easily observed in the early period after burn injury (15 minutes to 2 hours); however, venular contraction was observed after 5 hours. Cimetidine

Figure 4. Rolling leukocytes in rats receiving cimetidine or saline, 5 hours after the infliction of burn injury. * $P < 0.01$.

administration appeared to attenuate the microcirculatory disturbances when assessed after 5 hours.

In the present study, leukocyte adhesion in the venules of the stomach was suppressed with cimetidine after 5 hours. Administration of antineutrophil serum²¹ and monoclonal antibodies to various adhesion molecules^{21,22} was reported to inhibit gastric mucosal damage induced by aspirin. We have reported that rolling leukocytes and gastric mucosal lesions were interrelated.²³ We also have reported that adhesion molecule selectins played an important role in leukocyte adhesion in the gastric venules.²⁴ In that study, selectin antagonist attenuated not only the rolling of leukocytes but also gastric mucosal lesions. It is speculated in the present study that cimetidine directly affects attenuation of microcirculatory disturbance dependent on leukocytes.

This protective effect by cimetidine on gastric microcirculation may be a secondary effect caused by the suppression of acid secretion. However, the presence of direct effects of cimetidine on microcirculation was assumed because a previous report²⁵ revealed cimetidine-binding sites on the collecting venules of the stomach. Furthermore, it was reported that cimetidine scavenges AOS in vitro.^{26–29} Thus, we attempted to investigate the effect of this drug on scavenging AOS in vivo.

The mean ChL activities obtained from gastric veins in the cimetidine group were similar to those in the control group. This result does not disprove that

Figure 5. Luminol-dependent, zymosan-stimulated chemiluminescence (ChL) activity generated by leukocytes in gastric veins. Luminol-dependent, zymosan-stimulated ChL values from gastric veins were calculated.

cimetidine is one of the scavengers of AOS in vivo. Although a protective effect on gastric microcirculation was clearly seen, no statistically significant difference in ChL activities was found between the 2 groups in the present study. Factors in addition to the scavenging effect on AOS had to be considered for this microcirculatory protection.

CONCLUSION

The present study suggests that cimetidine may have a protective effect against gastric microcirculatory disturbances induced by burn injury, a model of surgical stress.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. Davenport HW. Salicylate damage to the gastric mucosal barrier. *N Engl J Med.* 1967; 276:1307-1312.

2. Kamada T, Sato N, Kawano S, et al. Gastric mucosal hemodynamics after thermal or head injury. A clinical application of reflectance spectrophotometry. *Gastroenterology*. 1982;83:535–540.
3. Kitajima M, Wolfe RR, Trelstad RL, et al. Gastric mucosal lesions after burn injury: Relationship to H⁺ back-diffusion and the microcirculation. *J Trauma*. 1978;18:644–650.
4. Yoshida M, Fukumura D, Wakabayashi G, et al. Gastric microcirculatory disturbance and behavior of leukocytes after thermal injury: Intravital observation of arterio-venous shunting channels in the gastric submucosal layer. *J Gastroenterol Hepatol*. 1995;10:365–370.
5. Arakawa T, Nakamura H, Chono S, et al. Difference in mode of action of cimetidine and gefarnate on endogenous prostacyclin, prostaglandin E₂ and thromboxane in rat gastric mucosa. *Tohoku J Exp Med*. 1983;40:407–412.
6. Tayama E, Hayashida N, Fukunaga S, et al. High-dose cimetidine reduces proinflammatory reaction after cardiac surgery with cardiopulmonary bypass. *Ann Thorac Surg*. 2001;72:1945–1949.
7. Hiramoto JS, Terdiman JP, Norton JA. Evidence-based analysis: Postoperative gastric bleeding: Etiology and prevention. *Surg Oncol*. 2003;12:919.
8. McElwee HP, Sirinek KR, Levine RA. Cimetidine affords protection equal to antacids in prevention of stress ulceration following thermal injury. *Surgery*. 1979;86:620–626.
9. Kitajima M, Ikeda Y, Ueda M, Sohma S. The microcirculation of the stomach in stress. In: Tsuchiya M, Asano M, Oda M, eds. *Basic Aspects of Microcirculation: Proceedings of the Tokyo International Symposium on Microcirculation, July 26, 1981*. Princeton, NJ: Excerpta Medica; 1982:230–242.
10. Wakabayashi G, Ueda M, Aikawa N, Abe O. Atrial natriuretic polypeptide after burn injury: Blood levels and physiological role in rats. *Burns*. 1990;16:169–175.
11. Carter EA, Gonnella A, Tompkins RG. Increased transcellular permeability of rat small intestine after thermal injury. *Burns*. 1992;18:117–120.
12. Yoshida M, Wakabayashi G, Otani Y, et al. Intravital observation of gastric microcirculatory disturbance induced by thermal injury. In: Tsuchiya M, Asano M, Tsushima N, eds. *Microcirculation Annual 1996*. Tokyo: Nihon-Igakukan; 1996:203–204.
13. Ohno T, Katori M, Nishiyama K, Saigenji K. Direct observation of microcirculation of the basal region of rat gastric mucosa. *J Gastroenterol*. 1995;30:557–564.
14. Rosenberg A, Guth PH. A method for the in vivo study of the gastric microcirculation. *Microvasc Res*. 1970;2:111–112.
15. Guth PH, Rosenberg A. In vivo microscopy of the gastric microcirculation. *Am J Dig Dis*. 1972;17:391–398.
16. Suematsu M, DeLano FA, Poole D, et al. Spatial and temporal correlation between leukocyte behavior and cell injury in postischemic rat skeletal muscle microcirculation. *Lab Invest*. 1994;70:684–695.
17. Yoshida M, Kitahora T, Wakabayashi G, et al. Active oxygen species in formation of acute gastric mucosal lesions induced by thermal injury in rats. *Dig Dis Sci*. 1995;40:1306–1310.
18. Kitahora T, Suzuki K, Asakura H, et al. Active oxygen species generated by monocytes and polymorphonuclear cells in Crohn's disease. *Dig Dis Sci*. 1988;33:951–955.
19. Kitajima M, Otsuka S, Shimizu A, et al. Impairment of gastric microcirculation in stress. *J Clin Gastroenterol*. 1988;10(Suppl 1):S120–S128.

20. Yoshida M, Wakabayashi G, Otani Y, et al. Active oxygen species generation by circulating leukocytes and gastric submucosal microcirculatory disturbances in the early period after thermal injury. *J Clin Gastroenterol*. 1995;21(Suppl 1):S87–S92.
21. Yoshida N, Yoshikawa T, Nakamura Y, et al. Role of neutrophil-endothelial cell interactions in gastric mucosal injury induced by aspirin. *J Clin Gastroenterol*. 1995; 21(Suppl 1):S73–S77.
22. Yoshida N, Yoshikawa T, Nakamura Y, et al. Role of neutrophil-mediated inflammation in aspirin-induced gastric mucosal injury. *Dig Dis Sci*. 1995;40:2300–2304.
23. Yoshida M, Wakabayashi G, Ishikawa H, et al. A protease inhibitor attenuates gastric erosions and microcirculatory disturbance in the early period after thermal injury in rats. *J Gastroenterol Hepatol*. 1998;13:104–108.
24. Ishikawa H, Yoshida M, Wakabayashi G, et al. Sialyl Lewis X analog attenuates gastric microcirculatory disturbance and gastric mucosal erosion induced by thermal injury in rats. *J Gastroenterol Hepatol*. 2003;18:47–52.
25. Nakamura M, Oda M, Kaneko K, et al. Radioautographic characterization of H₁ and H₂ receptor antagonists–binding sites in rat gastric mucosal microcirculatory system. In: Shu C, ed. *Vascular Endothelium in Health and Disease*. New York, NY: Plenum Press; 1988:151–160.
26. Lapenna D, De Gioia S, Mezzetti A, et al. H₂-receptor antagonists are scavengers of oxygen radicals. *Eur J Clin Invest*. 1994;24:476–481.
27. Okajima K, Koga S, Inoue M, et al. Inhibitory effect of cimetidine on reactive oxygen production by leukocytes and leukocyte aggregation [in Japanese]. *Igaku-no Ayumi*. 1989;149:69–70.
28. Kirkova M, Atanassova M, Russanov E. Effects of cimetidine and its metal complexes on nitroblue tetrazolium and ferricytochrome c reduction by superoxide radicals. *Gen Pharmacol*. 1999;33:271–276.
29. Lambat Z, Limson JL, Daya S. Cimetidine: Antioxidant and metal-binding properties. *J Pharm Pharmacol*. 2002;54:1681–1686.

Address correspondence to: Hideki Ishikawa, MD, PhD, Department of Emergency and Critical Care Medicine, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: hideki-i@sc.itc.keio.ac.jp