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Misoprostol But Not Antacid Prevents Endotoxin-Induced Gastric Mucosal Injury:

Role of Tumor Necrosis Factor—Alpha

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Many of the complications of septic shock are believed to be a consequence of elevated circulating levels of tumor necrosis factor (TNF), which is an important mediator of tissue injury. Prostaglandins (PGs) of the E series have recently been reported to inhibit TNF production in vitro. *We investigated the* in vivo *effect of misoprostol, a PGE_1 analog, on endotoxin-induced gastric mucosal injury and TNF production. For the gastric mucosal injury studies, groups of animals were pretreated with intragastric misoprostol (100 and 200 μg/kg) or with antacid (2 ml/animal of Maalox Plus) 30 min prior to a challenge with intravenous* E. coli *lipopolysaccharide (LPS) at 5.0 mg/kg. Stomachs were examined 3 hr after LPS. Systemic endotoxin alone induced microscopic edema, vascular congestion, and polymorphonuclear (PMN) infiltration of the gastric mucosa. Pretreatment with misoprostol, but not with antacid, significantly and dose-dependently reduced the gastric mucosal injury. For the TNF studies, groups of rats were given either misoprostol (100 or 200 μg/kg, intragastric), or saline 1 hr prior to LPS challenge. Serum samples were obtained 1.5 hr after LPS challenge. Misoprostol dose-dependently and significantly ($P < 0.01$) inhibited TNF activity. We conclude that misoprostol is a potent inhibitor of TNF systemic production and inhibits the gastric mucosal injury induced by endotoxemia. These studies suggest a potentially important therapeutic role for misoprostol in inflammatory diseases in which TNF exerts a contributory role.*

KEY WORDS: tumor necrosis factor; misoprostol; prostaglandins; endotoxemia; stress ulcers; antacids

Patients with multiorgan failure and sepsis are at great risk of increased mortality when complicated by gastrointestinal bleeding from stress ulcers (1, 2). Prostaglandins are known to prevent the damaging effects of various noxious agents and stress. While PGs have been shown to suppress gastric acid secretion and increase mucus and bicarbonate secretion (3–10), the exact mechanism of PG-mediated cytoprotection is still unclear (11, 12). Tumor necrosis factor-alpha (TNF-α) is an important mediator of endotoxin-induced tissue injury and is thought to be a proximal mediator of inflam-

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matory response that triggers the release of several mediators that act in cohort to initiate a large number of events leading to shock and tissue injury (13–19). The role of tumor necrosis factor (TNF) in the development of gastric mucosal injury is not known.

Prostaglandins of the E series have been shown to inhibit endotoxin stimulated release of TNF by peripheral blood mononuclear phagocytes isolated from systemic circulation *in vitro* (15, 20, 21). PGEs also have been shown to influence both humoral and cellular immune reactions in experimental animals and man. Endogenous PGEs act as a feedback inhibitor of many T-cell functions. Regulation of macrophage-derived tumor necrosis factor gene expression by PGE_2 also has been demonstrated, and it appears to inhibit TNF production at the level of transcription (20, 21). The aims of the study were twofold: (1) to investigate the effect of the PGE_1 analog misoprostol and high-dose antacid on gastric mucosal injury induced by endotoxin, and (2) to determine whether misoprostol could inhibit TNF activity in response to endotoxemia *in vivo*.

MATERIALS AND METHODS

Drugs. Misoprostol (200 μg tablet) was suspended in 5 ml water immediately prior to the experiment. The Maalox Plus antacid was purchased commercially. Each milliliter contained 90.0 mg of $Mg(OH)_2$, 100.0 mg of $Al(OH)_3$ and 8.0 mg of simethicone.

Procedure. Groups (N = 6–8) of fasted male Sprague-Dawley rats (240–350 g) were used throughout the studies. For the gastric mucosal injury study, the animals were randomized to the following treatment groups: (1) misoprostol 100 μg/kg, intragastric; (2) misoprostol 200 μg/kg, intragastric; (3) Maalox Plus liquid 2 ml/animal, intragastric; and (4) saline, intragastric.

All animals were pretreated with the above drugs 30 mins prior to the intravenous administration of 5.0 mg/kg of *E. coli* lipopolysaccharide (LPS). Three hours after the LPS challenge, the animals were sacrificed with an overdose of pentobarital sodium (Nembutal). The stomach was removed, opened along the lesser curvature, inspected for macroscopic lesions, and placed in formaldehyde for histologic study. The histologic studies were conducted by an investigator who was blinded to the treatment regimen. A single longitudinal slice from the section midway between the lesser and greater curvatures was made from each formalin-fixed stomach. The sections were stained with hematoxylin and eosin. Polymorphonuclear cells were counted on five subsequent high-power fields (40 × objective) in each section in the midbody of the stomach.

For the TNF studies, the animals were pretreated with misoprostol (100 and 200 μg/kg) or saline administered intragastrically 1 hr prior to the challenge with LPS (5.0 mg/kg, intravenously). Serum samples were obtained 1.5 hr after LPS challenge and frozen at −70° C. Previous studies from this laboratory had shown that TNF levels peak at 1.5 hr after LPS challenge and diminish within 3 hr (22). The serum TNF levels were determined by the L 929 cytotoxicity assay as previously described (22). The assay was verified by the addition of rabbit antibody against murine recombinant TNF-α (22). Statistical analysis was performed using the analysis of variance (ANOVA) method.

RESULTS

Effect of Treatment on Gastric Mucosal Injury. There were no gross intraluminal bleeding or macroscopic lesions observed when the stomachs were examined in all groups. However, on histologic examination, the groups treated with LPS alone exhibited mucosal and submucosal edema, vascular congestion, and PMN infiltration (Figures 1 and 2). Few shallow erosions were confined within the epithelial layer. Both doses of misoprostol induced profound attenuation of mucosal edema, vascular congestion, and PMN infiltration. The mucosal and submucosal PMN cell counts were significantly ($P < 0.01$) and dose-dependently reduced by misoprostol (Table 1). In contrast, there was no significant effect on mucosal and submucosal PMN counts achieved by the antacid treatment (Table 1).

Effect on TNF Production. The administration of LPS significantly elevated serum TNF levels (Table 2). TNF was not detected in the sera of rats not challenged with LPS. Misoprostol significantly ($P < 0.01$) and dose-dependently reduced TNF levels in response to the endotoxin (Table 2).

DISCUSSION

By some estimates, septic shock is presently the commonest cause of death in intensive care units in the United States (23). The basis for this increased incidence is not fully understood but appears to be of a multifactorial origin (24). While many therapies have been advocated for the treatment of septic shock, none of these therapies has been found to alter its catastrophic course. These therapies includes the use of sympathomimetics, endorphin antagonists (eg, naloxone), and antihistamines (24).

Recent evidence indicates that cytokines play a major role in the host response to septicemia (25). Endotoxin-associated tissue injury, including PMN cell infiltration, appears to be mediated by TNF release (13–19). TNF is thought to be a proximal mediator of the inflammatory response and triggers

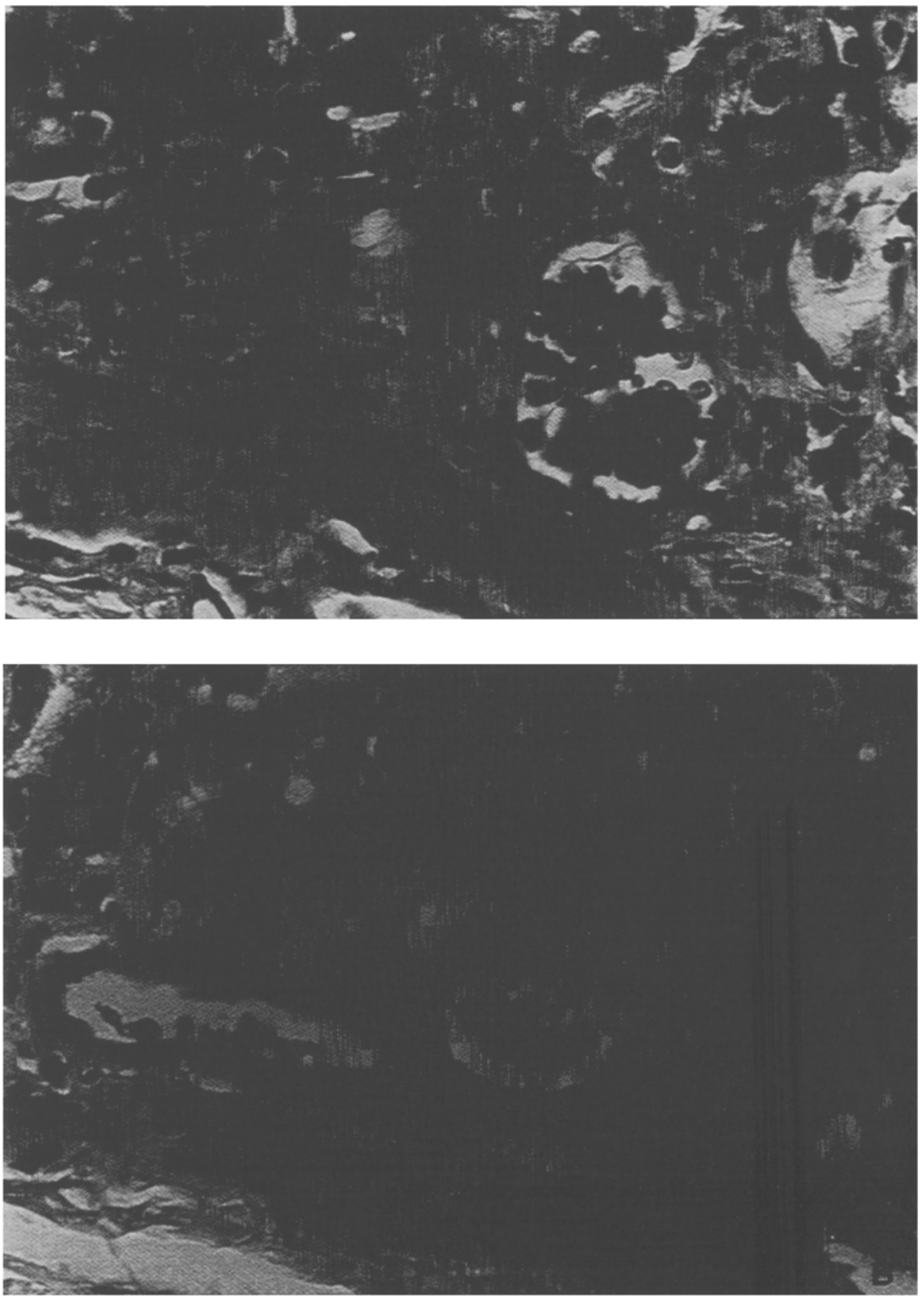

Fig 1. (A) Gastric mucosal morphology of control animal treated with LPS alone (H&E, ×400). Note prominence of PMN infiltrates and vascular congestion. (B) Gastric mucosal morphology of an animal pretreated with 200 μg/kg of misoprostol (H&E, ×400). Note absence of PMN infiltrates and vascular congestion.

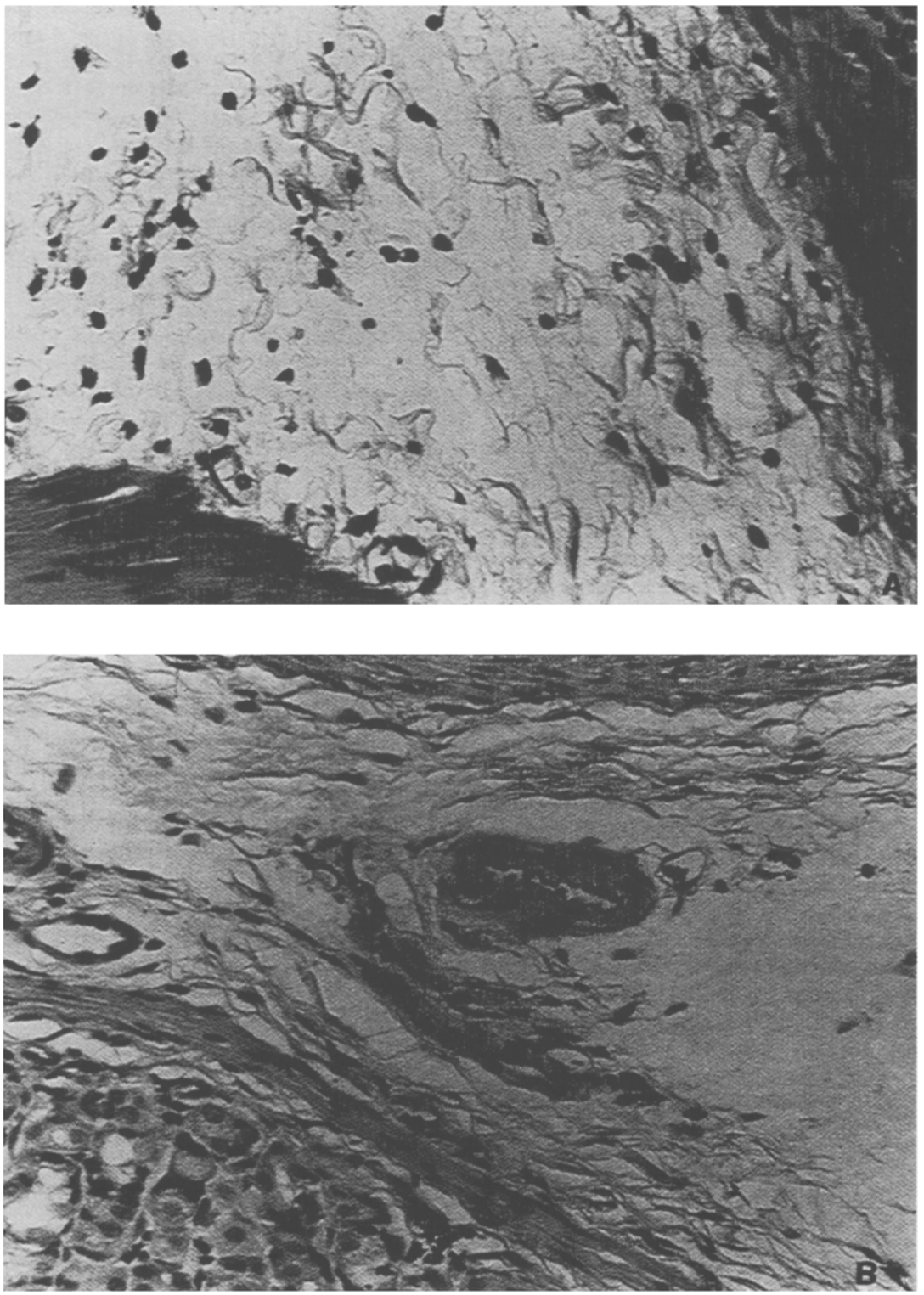

Fig 2. (A) Gastric submucosal morphology of a control animal treated with LPS alone (H&E, ×200). Note prominence of PMN infiltrates. (B) Gastric submucosal morphology of an animal treated with 200 μg/kg of misoprostol (H&E, ×200). Note the absence of PMN infiltrates and vascular congestion.

TABLE 1. EFFECT OF MISOPROSTOL AND CONCENTRATED ANTACID ON LPS-INDUCED GASTRIC PMN CELL INFILTRATION*

	PMN infiltration (±SEM)/5	*HPF (40× Obj)*
Parameter	*Mucosa*	*Submucosa*
Saline	88.5 ± 9.9	31.0 ± 6.0
Misoprostol		
200 μg/kg	19.5 ± 2.4a†	7.5 ± 2.2b
100 μg/kg	45.1 ± 5.6b	4.3 ± 1.2b
Maalox Plus (2 ml/animal)	79.5 ± 11.1 NS	29.8 ± 8.5 NS

*Groups of fasted rats were pretreated with either misoprostol, or saline or antacid 30 min prior to the induction of endotoxemia (*E. coli* LPS at 5 mg/kg, intravenous). The animals were sacrificed 3 hr later, the stomachs were fixed, and PMN cells were counted.
†a, $P < 0.001$; b, $P < 0.01$ compared with saline.

the release of several mediators that act to initiate a large number of events leading to tissue injury, multiorgan failure, and shock. TNF has been known to activate PMN functions and to enhance their phagocytic and bactericidal activities (16, 19). It also appears that TNF is directly toxic to vascular endothelial cells. Hemorrhagic necrosis may occur in normal tissues in animals treated with sublethal doses of TNF (18). We believe that this is the first study demonstrating that misoprostol significantly inhibits TNF production *in vivo* and prevents acute inflammation in the gastric mucosal tissue in rats. Misoprostol was also reported to inhibit the endotoxemia-induced gastric mucosal injury in cats, and this protective action was not mediated by alteration of gastric mucosal hemodynamics (26, 27). In a prospective double-blind clinical study, misoprostol was also found to be as effective as titrated antacids in preventing clinically evident upper gastrointestinal hemorrhage and upper gastrointestinal lesions in postsurgical patients managed in intensive care units (28). The question of the role of TNF in the induction of gastric mucosal damage has not been adequately studied. However, a recent study by Kahky et al (29) disclosed that the systemic (portal vein) administration of TNF induced systemic gastric and small intestinal mucosal injury, pulmonary edema, and acute tubular necrosis.

TABLE 2. EFFECT OF MISOPROSTOL ON SERUM LEVELS OF TNF-α IN LPS-TREATED RATS*

Groups	*Serum TNF (units/ml)*
Saline	13,202 ± 606
Misoprostol	
100 μg/kg	6,038 ± 258†
200 μg/kg	2,915 ± 189†
Control (no LPS)	Not detected

*Groups of fasted rats received either misoprostol or saline 1 hr prior to LPS administration (*E. coli* LPS at 5.0 mg/kg intravenous). Serum samples were assayed for TNF levels at 1.5 hr following LPS challenge.
†$P < 0.01$ compared with saline.

The present studies were carefully designed to examine two consequences of endotoxemia, namely gastric mucosal injury and TNF production as the possible mechanisms that mediate this injury. The 90-min time period taken for the measurement of TNF serum level represented the peak period for this cytokine (22). The question of whether misoprostol treatment could have delayed the appearance of TNF peak is an important but difficult issue to resolve. This difficulty relates to the fact the TNF is a "hit-and-run" cytokine, and many time points would need to be measured over the course of many hours. Future studies should also examine the pharmacokinetics of TNF, especially in response to the treatment with either intravenous and/or intragastric misoprostol. The intravenous route of administration of misoprostol has a superior systemic bioavailability and hence should possess greater efficacy in endotoxemia. In fact, a recent study reported increased survival in cats against experimentally induced endotoxemia with intravenous misoprostol (26). Future studies should also investigate whether the neutralization of TNF by TNF antibody or the reduction of TNF serum level by antagonists of platelet-activating factor (30) would produce similar results as those observed with misoprostol.

Considerable pharmacological evidence indicates that gastric acid plays an important role in the induction of stress ulcer in animals (31, 32). However, the role of gastric acid in the endotoxemia-induced mucosal injury is not well known. In the present studies, misoprostol was tested at doses that do not inhibit gastric acid secretion (33, 34). Thus, the mucosal protective action of misoprostol against the LPS-induced gastric mucosal injury appears to be mediated by a mechanism not related to the inhibition of gastric acid. The inactivity of the high-dose antacid tested in the present study further supports the notion that gastric acid is not important in the etiology of LPS-induced gastric mucosal injury. Thus the potential use of misoprostol in the prophylaxis of stress ulcer may have additional benefits, particularly in endotoxemia.

The immunomodulating action of E prostaglandins against TNF may also be significant in some

disease states where there is significant elevation of this cytokine. Preliminary unpublished experience from this laboratory also suggests that misoprostol is effective in preventing the endotoxin-induced sequestration of PMNs in the pulmonary vasculature. There are now several clinical studies that implicate a contributory role of TNF in cachexia (25, 35), end-stage heart failure (36), cerebral malaria (37), leprosy (38), parasitic infections (39), lupus erythematosus (40), rheumatoid arthritis (41), cancer (42) and acquired immunodeficiency syndrome (43). Misoprostol and other PGEs could prove to be of value in treating these conditions due to their inhibitory effects on TNF and possibly on other cytokines. Prospective controlled preclinical and clinical studies with misoprostol in these disease states are now warranted.

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