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Indomethacin potentiates endotoxin-induced blood flow reduction and histological injury in rat gastric mucosa

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1 The effect of the intravenous administration of lipopolysaccharide from *Salmonella typhosa* endotoxin on arterial blood pressure (BP), gastric mucosal blood flow (GMBF) and gastric damage was studied in anaesthetized rats. The effect of the inhibition of endogenous prostaglandin generation by indomethacin on these parameters was also investigated in this model of endotoxin shock.

2 A similar and dose-dependent percentage of reduction in BP and GMBF was observed 5 min after a bolus injection of 20 or 30 mg kg^{-1} endotoxin. A transient recovery in GMBF at 15 min was observed followed by a second fall at 30 min, at a time when BP was slowly increasing.

3 Pretreatment with indomethacin (5 mg kg^{-1}, s.c.) one hour before the administration of 30 mg kg^{-1} endotoxin, significantly augmented the reduction in GMBF without affecting the reduction in BP.

4 The gastric damage, assessed histologically, was similar and confined to the superficial mucosa 30 min after the administration of 20 or 30 mg kg^{-1} endotoxin. The histologically-assessed damage was significantly greater in indomethacin pretreated rats injected with 30 mg kg^{-1} endotoxin.

5 These findings suggest that endogenous prostaglandin generation plays a protective role in endotoxin-induced gastric mucosal microcirculatory disturbances and mucosal damage.

Introduction

Endotoxin infusion, either with (Wallace & Whittle, 1986) or without (Cheung *et al.*, 1975) systemic hypotension, leads to gastric mucosal damage in different experimental models. The pathophysiology of this mucosal injury is not well known. It has been suggested that mucosal ischaemia may play a pathogenic role in the development of gastric damage in endotoxaemia (Cheung *et al.*, 1976) and such ischaemia may be brought about by systemic hypotension and/or the release of endogenous mediators. Thus, release of the low molecular weight phospholipid, platelet-activating factor (Paf) has been implicated as a mediator of the gastrointestinal damage that accompanies endotoxin shock (Rosam *et al.*, 1986; Whittle *et al.*, 1987). Studies of the rat gastric microcirculation have demonstrated that Paf induces sluggish blood flow in the submucosal vessels and stasis in the mucosal capillaries (Whittle *et al.*, 1986).

Release of several prostanoids, including thromboxane, has been demonstrated in endotoxin shock in different species (Anderson *et al.*, 1975; Cook *et al.*, 1980), but their pathophysiological role remains unclear (Fletcher & Ramwell, 1980a). The inhibition of prostaglandin synthesis by cyclo-oxygenase inhibitors can improve systemic haemodynamics in experimental endotoxin shock in some models (Cook *et al.*, 1980; Fletcher & Ramwell, 1980b), but certain prostaglandins, such as prostacyclin, appear to exert a protective effect against endotoxin-induced injury in the lung (Demling *et al.*, 1981).

In the present study, we have examined the effect of the intravenous administration of endotoxin from *Salmonella typhosa* on gastric mucosal blood flow and gastric damage in anaesthetized rats. The effect of the inhibition of endogenous prostaglandin formation by indomethacin on systemic arterial blood pressure, mucosal blood flow and gastric damage in this model has also been determined. The data are

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used to test the hypothesis that endogenous prostaglandin generation plays a protective role in endotoxin-induced gastric mucosal microcirculatory disturbance and histological damage.

Methods

Animal preparation

Male Wistar rats, weighing 200–275 g were fasted for 24 h before the experiments but allowed free access to water. The rats were anaesthetized with pentobarbitone sodium (50 mg kg^{-1}, i.p.). A tracheostomy was performed and a polyethylene tubing (PE-250) was inserted into the trachea to facilitate spontaneous breathing. The right carotid artery was cannulated with PE-90 tubing for blood pressure monitoring. A needle (23 gauge) attached to a PE-50 tubing was inserted into a femoral vein for the administration of saline during the resting period or of endotoxin during the study period. The abdomen was opened via a mid-line incision to expose the stomach, and the gastric contents washed out with isotonic saline through an incision in the forestomach. The rat was kept warm with a heat lamp, maintaining rectal temperature at 37°C.

Hydrogen-gas clearance

An incision was made through the serosa and the muscularis externa of the gastric wall of the corpus, exposing 3–4 mm of the submucosa. Through this hole, a platinum electrode was placed in contact with the exposed basal portion of the mucosa. A reference electrode was placed in the peritoneal cavity. The laparotomy incision was covered by Parafilm to minimize evaporation.

The hydrogen-gas clearance technique for the measurement of gastric mucosal blood flow has been described and validated in previous studies (Leung *et al.*, 1984; Cheung & Sonnenschein, 1984; Scremin *et al.*, 1987). When the experimental animal breathes 3% hydrogen in air, the tracing of the current generated at the surface of the platinum electrode and graphed on a recorder (Gilson Medical Electronics, Middleton, Wish), gradually rises and reaches a plateau within a 15 min period, indicating saturation of the gastric mucosa with hydrogen. This current, which is measured with a polarographic and amplifying unit (Val Tech Electronics, Sherman Oaks, CA), is proportional to the hydrogen tension gradient at the surface of the platinum electrode. After the external hydrogen source is removed, the current tracing gradually falls due to removal of tissue hydrogen by blood flow. Current from the platinum electrode is passed to an ADALAB analog to digital converter and discrete digitized values are sampled every 5 s. Hydrogen gas (3% in air) was administered to the rat for 15 min, with the desaturation phase being started 5 min after the administration of endotoxin.

The clearance curves were analyzed by use of a recently described non-linear least square fit computer programme (Livingston *et al.*, 1986), and gastric mucosal blood flow was expressed in ml min^{-1} 100 g^{-1} of tissue.

In vivo *microscopy technique*

An *in vivo* microscopy technique was used to study the superficial gastric mucosal microcirculation (Guth & Rosenberg, 1972). The anaesthetized rats were placed on a stage on a heating pad. The abdomen was opened and an incision was made in the anterior wall of the forestomach. The posterior wall of the corpus mucosa was then everted through the incision. A fibreoptic light carrier rod was placed beneath the serosa of the everted corpus. The exposed mucosa was continuously superfused with Krebs solution (pH 7.4, 37.5°C) to maintain hydration and temperature.

An American Optical Microstar microscope with a Leitz long working distance 32 × /0.4 objective was used to visualize blood flow through the superficial microvessels of the exposed mucosa. The microscope was connected to a closed circuit television system which consisted of a video camera (Cohu 4400), a television screen (Hitachi VM-129U) and a videotape recorder (JVC HR-7100U). The final magnification on the television screen was 750 ×. All *in vivo* microscopic studies were videotaped for later analysis of red blood cell velocity (RBCV).

Red blood cell velocity (RBCV) was measured in first-order post-capillary venules (diameter 12–20 μm) by a technique previously described (Holm-Rutili & Obrink, 1985). For the determination of RBCV, the voltage output at two points in a microvessel was monitored on the playback of the videotape, and the signals were fed into a Red Blood Cell Velocity Tracking Correlator (Instrumentation for Physiology and Medicine, San Diego). The voltage varies as red blood cells pass each point, and the correlator can determine the time lag of similar patterns between the upstream and downstream signals. By continuously dividing this time lag into the present distance between the two points, the correlator can continuously compute the RBCV. The lowest detectable level of RBCV in this system is 0.05 nm s^{-1}. One vessel in each preparation, based on its appearance in the control period, being in clear focus and straight, was selected for study. The vessel was followed throughout the experiment, with centreline velocity being measured for 1 min periods.

Experimental design

Dose-related effects of endotoxin on arterial blood pressure and mucosal blood flow, measured by hydrogen-gas clearance During the first 30 min of each experiment, basal mucosal blood flow was measured in all the rats. After this period, a bolus of $20\,mg\,kg^{-1}$ or $30\,mg\,kg^{-1}$ lipopolysaccharide from *Salmonella typhosa* endotoxin (Sigma Chemical Co., lot 93F-4021) dissolved in saline, was injected intravenously over a 1 min period in separate groups of 6 animals each. The mucosal blood flow measurement was then repeated. Since arterial blood pressure fell during the first 5 min after endotoxin administration but then remained at a low level with only slight recovery, the desaturation phase of the hydrogen gas clearance (cessation of hydrogen administration) was not started until 5 min after endotoxin administration.

Systemic arterial blood pressure was monitored throughout the experiment. The mean blood pressure during the 15 min desaturation period of each of the two blood flow measurements (basal and endotoxin periods) was calculated. Results of mucosal blood flow and arterial blood pressure after endotoxin administration are expressed as percentage of baseline values.

Effect of indomethacin pretreatment on arterial blood pressure and mucosal blood flow during endotoxin shock In a different group of rats ($n = 7$) receiving $30\,mg\,kg^{-1}$ endotoxin, the same procedures were performed, but indomethacin ($5\,mg\,kg^{-1}$) was injected subcutaneously 1 h before the start of the experiments.

Effect of endotoxin, with or without indomethacin pretreatment, on histological injury The rats were killed 30 min after the endotoxin administration in each of the above two series of experiments, and the stomach was opened along the greater curvature. A strip of tissue from across the posterior wall was excised, fixed, embedded in paraffin, sectioned and stained with haematoxylin and eosin by routine histological procedures.

Histological tissue sections from all the groups were coded, randomized, and examined for gastric corpus mucosal injury by an observer who was unaware of the treatments. The severity of gastric mucosal injury was evaluated according to a recent published modification (Itoh & Guth, 1986) of the criteria previously described (Lacy & Ito, 1982). Damage of the corpus mucosa was graded as follows: (0)—all gastric mucosal cells appeared intact and had normal shape, location, appearance, and density; (1)—surface mucous cell damage: these cells were vacuolated, had pyknotic nuclei and brightly stained or lysed cytoplasm; (2)—extensive surface cell damage plus disruption and exfoliation of cells lining the gastric pits (in some areas, the first or second parietal cells lining the glands were also involved); (3)—damage extending beyond the gastric pits but involving $<50\%$ of the thickness of the gastric mucosa; (4)—extensive gastric mucosal damage involving $>50\%$ of the thickness of the gastric mucosa.

By use of an ocular micrometer, the length of each grade of cellular damage and the section length were determined for each specimen. The percentage of the mucosal length with each grade of damage was then calculated. In order to determine the average grade of damage in each section, the histological index was calculated by multiplying the percentage length of each grade of damage by 1, 2, 3 and 4 respectively, and dividing the result by 100. The resulting index represents the average grade of damage in each section.

Time-course relationship between arterial blood pressure and superficial gastric mucosal blood flow after endotoxin administration *In vivo* microscopy studies were performed in 6 rats. Arterial blood pressure and RBCV were measured during the basal period and for 30 min after $30\,mg\,kg^{-1}$ endotoxin administration. For data analysis, every 5 min, arterial blood pressure and RBCV measurements were compared with baseline values.

Data analysis

All data are expressed as mean $\pm$ standard error (s.e.). For statistical evaluations of differences paired or unpaired Student's t tests or ANOVA with contrasts were used. A probability level of $P < 0.05$ was considered significant.

Results

There were no differences in basal arterial blood pressure or mucosal blood flow among the three groups of animals (Table 1).

Dose-related effects of endotoxin on blood pressure and mucosal blood flow

A significant reduction of arterial blood pressure (BP) and gastric mucosal blood flow (GMBF) measured by hydrogen-gas clearance was observed 5–20 min after endotoxin administration (Table 1). The percentage of reduction in BP and GMBF was similar and dose-dependent, being 24% and 29%, respectively with $20\,mg\,kg^{-1}$ endotoxin and 53% and 55% respectively, with $30\,mg\,kg^{-1}$ endotoxin.

Table 1 Endotoxin-induced haemodynamic changes with and without inhibition of endogenous prostaglandin synthesis

Endotoxin ($mg\,kg^{-1}$)	*Basal* BP	*Basal* GMBF	*Study* BP	*Study* GMBF	(n)
20	118 ± 6	34 ± 4	91 ± 8*	24 ± 4*	(6)
30	121 ± 4	38 ± 5	57 ± 7***	17 ± 2**	(6)
30 + Indomethacin	115 ± 5	39 ± 4	54 ± 7***	12 ± 2***	(7)

Arterial blood pressure (BP, mmHg) and gastric blood flow (GMBF, $ml\,min^{-1}\,100\,g^{-1}$) measured by hydrogen gas clearance, during basal conditions (basal) and the study period (after endotoxin administration), with 20 or $30\,mg\,kg^{-1}$ endotoxin administration and $30\,mg\,kg^{-1}$ endotoxin in indomethacin ($5\,mg\,kg^{-1}$) pretreated rats. Results are shown as mean ± s.e. of (*n*) experiments. Significant difference from basal periods (BP and GMBF): * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

Effect of indomethacin pretreatment on blood pressure and mucosal blood flow during endotoxic shock

Pretreatment with indomethacin ($5\,mg\,kg^{-1}$, s.c.) did not produce any significant change in basal GMBF or BP, or in fall in BP after $30\,mg\,kg^{-1}$ endotoxin as compared with untreated rats (Table 1). The reduction in GMBF after $30\,mg\,kg^{-1}$ endotoxin was, however, significantly greater ($P < 0.01$) in indomethacin pretreated rats (Figure 1).

Effect of endotoxin, with or without indomethacin pretreatment, on histological injury

The damage to the gastric mucosa, as assessed histologically, was confined to the superficial mucosa and consisted of mucous cell damage and areas of vasocongestion. There was no significant difference in gastric damage, measured by the histological index, between rats injected with 20 or $30\,mg\,kg^{-1}$ endotoxin (Figure 2). The histological index was significantly ($P < 0.05$) higher however, in the rats receiving endotoxin ($30\,mg\,kg^{-1}$) that had been pretreated with indomethacin (Figure 2). The injury involved the neck cells and extended into the parietal cell region in some areas.

Time-course relationship between arterial blood pressure and superficial gastric mucosal blood flow after endotoxin administration

In this series of studies, the basal value for BP was 106 ± 4 mmHg and RBCV was $1.2 \pm 0.1\,mm\,s^{-1}$.

A parallel fall in BP and RBCV in the vessels of the superficial mucosa was observed 3–5 min after $30\,mg\,kg^{-1}$ endotoxin administration (Figure 3). Thereafter, BP showed a small but significant recovery so that at 30 min, BP was significantly ($P < 0.05$) higher than the lowest value reached at 5 min (74 ± 5 compared with 61 ± 5 mmHg, respectively). In contrast, RBCV showed a significant ($P < 0.05$) transient recovery so that at 15 min, RBCV was 0.95 ± 0.1 compared with 0.67 ± 0.1 $mm\,s^{-1}$ at 5 min. However, this was followed by a

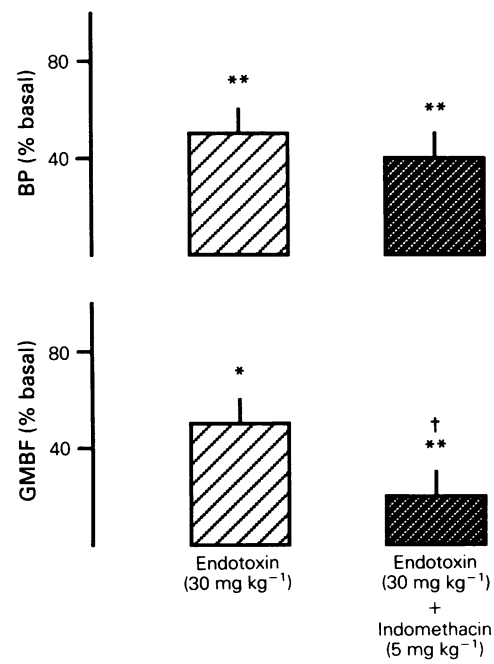

Figure 1 Arterial blood pressure (BP) and gastric mucosal blood flow (GMBF), expressed as percentage of basal values, 5–20 min after $30\,mg\,kg^{-1}$ endotoxin administration in rats with and without pretreatment with $5\,mg\,kg^{-1}$ indomethacin. Each column represents the mean of six experiments; vertical lines show s.e. mean. Significant difference from basal values: * $P < 0.01$; ** $P < 0.001$. Significant difference from group of rats not treated with indomethacin: † $P < 0.05$.

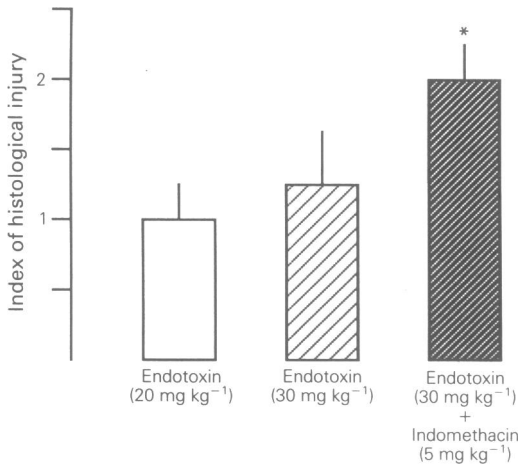

Figure 2 Histological injury assessed in the corpus gastric mucosa of rats 30 min after administration of 20 or 30 mg kg^{-1} endotoxin and in rats pretreated with 5 mg kg^{-1} indomethacin 1 h before the administration of 30 mg kg^{-1} endotoxin. The results are shown in terms of histological index and each column represents the mean of 6 experiments; vertical lines show s.e. mean. * Significantly higher than 20 mg kg^{-1} endotoxin group: ($P < 0.01$) and 30 mg kg^{-1} endotoxin group ($P < 0.05$). The index values of 1.0 and 1.3 in the non-indomethacin pretreated rats indicates an average grade 1 or surface mucous cell injury. The index value of 2.0 in indomethacin pretreated rats indicates an average grade 2 or neck cell injury.

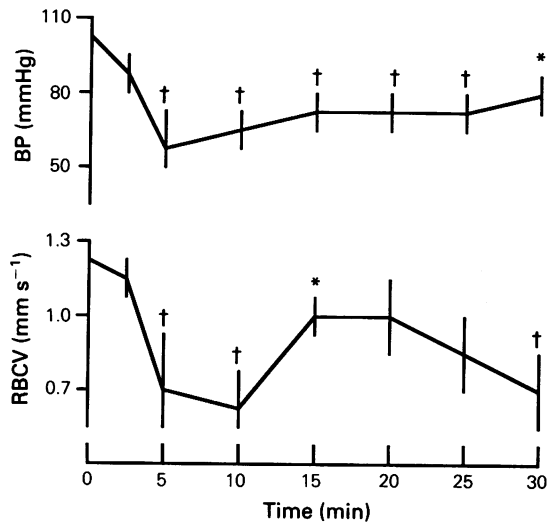

Figure 3 Systemic arterial blood pressure (BP) and red blood cell velocity (RBCV) measurements every 5 min after administration of 30 mg kg^{-1} endotoxin. Each point represents the mean of 6 experiments with s.e. shown by vertical lines. Significantly different from baseline values (ANOVA with contrasts): †$P < 0.05$. Significantly higher than mean value at 5 min (BP) and 10 and 30 min (RBCV): *$P < 0.05$.

second significant ($P < 0.05$) gradual fall in RBCV to 0.73 ± 0.1 mm s^{-1} at 30 min.

Discussion

Acute gastric ulceration and diffuse bleeding may occur following various clinical situations, including septic shock (Silen *et al.*, 1981). Gastric damage has also been documented experimentally with endotoxin-induced sepsis (Wallace & Whittle, 1986) or septic shock (Cheung *et al.*, 1975). The importance of mucosal blood flow in the defence of the gastric mucosa against injury has been demonstrated in haemorrhagic shock models (Starlinger *et al.*, 1981; Leung *et al.*, 1985). Increased gastric blood flow has been reported in studies using live organisms to induce bacteriaemia (Nilsson *et al.*, 1983; Genter *et al.*, 1983), but a decreased gastric blood flow has been reported with intra-arterial infusion of endotoxin even in the absence of systemic hypotension (Cheung *et al.*, 1976). In the present study, where endotoxin shock was induced by lipopolysaccharide from *Salmonella typhosa*, a dose-related and parallel reduction in arterial blood pressure and gastric mucosal blood flow measured by hydrogen-gas clearance, was observed during the 15 min period of measurement after endotoxin administration. This almost identical reduction in blood pressure and blood flow along with the *in vivo* microscopy observation that the fall in red blood cell velocity in the superficial microvessels started simultaneously with the fall in blood pressure, suggests that in this early period the depression in blood flow was probably a consequence of the effect of the endotoxin on systemic haemodynamics. It is relevant that intravenous infusion of Paf, a putative mediator of endotoxin shock (Rosam *et al.*, 1986; Whittle *et al.*, 1987) also causes a substantial fall in arterial blood pressure as well as in gastric mucosal blood flow measured either by hydrogen gas clearance or *in vivo* microscopy (Whittle *et al.*, 1986). In such doses, Paf-induced mucosal damage was characterized predominantly by diffuse vasocongestion and epithelial damage (Rosam *et al.*, 1986; Wallace *et al.*, 1986). In the present study, 30 min after endotoxin administration, a dissociation between the actions on blood pressure and blood flow was observed by *in vivo* microscopy. There was a significant recovery in arterial blood pressure compared with the lowest value reached at 5 min after endotoxin administration. In contrast, red blood cell velocity fell again after a marked transient recovery at 15 min, while

blood pressure slowly increased to a similar level to that observed during the initial fall. This later decrease in red blood cell velocity in the superficial mucosa despite the recovery in arterial blood pressure, might be explained by the subsequent local release of mediators induced by endotoxin or as a consequence of the superficial mucosal damage.

In spite of a significantly greater reduction in blood flow and blood pressure with the higher dose of endotoxin ($30\,mg\,kg^{-1}$), the histologically assessed injury to the mucosa was not significantly higher than that observed with endotoxin $20\,mg\,kg^{-1}$. This injury was confined to the very superficial mucosa (type 1 damage) and consisted of surface mucous cell damage and areas of vasocongestion. These findings are in agreement with those previously reported (Leung *et al.*, 1985) in a model of experimental damage following haemorrhagic shock plus intragastric HCl, where only mild damage was observed when the reduction in blood pressure and mucosal blood flow was less than 30–40%. In that study, systemic blood pressure and mucosal blood flow had to be reduced to 25% or less of the baseline value before severe and extensive lesions appeared. In the current study, macroscopic examination of the gastric mucosa in animals pretreated with indomethacin prior to endotoxin administration showed a more marked vasocongestion than in rats receiving the same dose of endotoxin alone. Likewise there was a significantly greater histologically assessed injury observed in the animals pretreated with indomethacin prior to endotoxin. Although pretreatment with indomethacin did not augment significantly the fall in blood pressure that followed administration of endotoxin, the reduction in gastric mucosal blood flow was significantly greater in such pretreated animals. These findings support the hypothesis that endogenous prostaglandin generation plays a protective role in endotoxin-induced gastric mucosal microcirculatory disturbance and gastric mucosal damage.

In the present study, indomethacin did not significantly reduce basal mucosal blood flow, perhaps indicating that endogenous prostanoids are less involved in the modulation of the microcirculation under resting conditions than under pathological situations. However, in a previous study with a higher dose of indomethacin ($10\,mg\,kg^{-1}$), a significant reduction in the resting vessel diameter in the arterioles of the rat gastric submucosa was observed (Guth & Moler, 1981), suggesting that a near-maximal degree of prostanoid inhibition is required to affect the microvasculature under resting conditions. In support of these findings, systemic administration of prostaglandin E_2 reduces the gastric mucosal damage induced by endotoxin in the rat (Wallace & Whittle, 1987). The mechanism of this protective effect of exogenous prostanoids against endotoxin-induced damage is not known. In addition to an increase in blood flow, it might reflect an inhibition of the local or systemic release of cellular damaging mediators such as Paf, lysosomal enzymes or free radicals. Whether inhibition of the biosynthesis of endogenous prostanoids would lead to a greater release of such tissue destructive mediators following endotoxin is not yet known. Identification of the endogenous mediators released in endotoxin shock and their pathophysiological actions on cardiovascular and microcirculatory systems and tissue integrity should provide new therapeutic approaches to the prevention of gastrointestinal damage associated with endotoxin and septic shock.

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