Indomethacin and the Gastric Mucosal Blood Flow Changes of Sepsis

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Recent evidence suggests that sepsis results in increased gastric mucosal blood flow (GMBF). To investigate the possible role of prostaglandins in mediating this response, the GMBF was measured in the fundus, corpus, and antrum of pig stomachs with and without pretreatment with indomethacin, an inhibitor of prostaglandin synthesis, before and after the induction of bacteremia. The studies were done in 22 piglets (seven sepsis controls, seven indomethacin controls and eight experimental [indomethacin pretreated sepsis]). Sepsis was produced in piglets by bolus intravenous injection of 10^9 live *Escherichia coli* followed by an infusion of 10^9 *E. coli*/hr. Cardiac output (C.O.) was measured by thermodilution. GMBF was measured by microsphere trapping. Following sacrifice, hyperemia was noted in the sepsis group but not in the other groups. GMBF was determined by standard techniques (expressed as ml/min/100 gm tissue). There were significant ($p < 0.05$) increases in gastric mucosal blood flow to the fundus (+47%), corpus (+50%), and antrum (+101%) at 15 minutes following the onset of *E. coli* infusion. At 135 minutes, the increase was only significant in the antrum. GMBF, however, did not change in the indomethacin control or indomethacin pretreated sepsis groups. These data demonstrate ↑ GMBF in the stomach following sepsis. The changes were not present in the indomethacin control or in the indomethacin pretreated sepsis groups. Since indomethacin is an inhibitor of prostaglandin synthesis, the results suggest that the ↑ GMBF may be a prostaglandin mediated response.

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SEPSIS FREQUENTLY LEADS TO gastric mucosal injury and stress bleeding. The pathophysiology leading to mucosal injury is controversial. Several studies of the effect of sepsis on intragastric blood flow using endotoxin models have shown decreased total gastric blood flow and decreased gastric mucosal blood flow.[1,2] The use of endotoxin models of sepsis to study gastric physiology have now been abandoned because of depression of the systemic circulation, which leads to secondary decreases in gastric blood flow.[3] Lucas et al.[4] have reported increased total gastric blood flow proportional to increases in cardiac output using a piglet septic hindlimb preparation. Our own group[5] has demonstrated increased total gastric blood flow and increased gastric mucosal blood flow in piglets following live bacteremia. It has been suggested that changes in gastric mucosal blood flow are mediated by prostaglandins.[5,6] In order to investigate the possible role of prostaglandins in the gastric blood flow changes of sepsis, septic piglets were studied following treatment with indomethacin, an inhibitor of prostaglandin synthesis.

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Methods

Twenty-two piglets (seven Sepsis controls, seven Indomethacin controls, and eight experimental [indomethacin pretreated sepsis]) of mean body weight 10 kg were used for the experiment. All animals were free of disease and housed in our animal unit for at least 48 hours prior to the experimental protocol. The protocol was essentially the same in the three groups.

The animals were anesthetized with pentobarbital sodium (30 mg/kg) administered intraperitoneally. After tracheostomy, they were ventilated with room air. Appropriate plastic cannulae were placed in the femoral artery for blood pressure monitoring and blood sampling and in the femoral vein for infusions and bacterial inoculation. A Swan-Ganz catheter was introduced into the pulmonary artery for cardiac output computation. Cardiac output was determined by thermodilution using a KMA cardiac output computer. Core body temperature was recorded electrically via thermistor. An additional catheter was introduced into the left ventricle via the left carotid artery for microsphere injections.

Following anesthesia, the animals were allowed to stabilize for 30 minutes. An infusion of 0.45% sodium chloride solution was started and administered at a constant rate of 2 ml/min throughout the course of the experiment. Following stabilization, the baseline intragastric blood distribution was marked by the intraventricular injection of approximately 600,000 15μ diameter plastic radioactive microspheres labelled with Sr^{85}, Ce^{141}, Cr^{51}, or I^{125}. The

TABLE 1. *Core Temperature (°C)*

	Time (min)		
	0	15	135
Escherichia coli bacteremia	36.1	36.1	37.9*
Indomethacin control	35.2	35.0	34.8
Indomethacin pretreated bacteremia	36.1	36.1	37.7*

* Significance ($p < 0.05$).

TABLE 2. *Cardiac Output (l/min)*

	Time (min)		
	0	15	135
Escherichia coli bacteremia	1.68	1.68	1.79
Indomethacin control	1.74	1.59	1.43
Indomethacin pretreated bacteremia	1.53	1.53	1.83

choice of radioactive marker was made randomly with the remaining isotopes reserved for the latter parts of the experiment.

Immediately after baseline measurements were complete, indomethacin (2 mg/kg) was administered intravenously over 10 minutes. Following completion of the indomethacin infusion, bacteremia was produced by a bolus intravenous injection of 10^9 live *Escherichia coli* (strain U1036) followed by infusion of 10^9 bacteria/hour during the rest of the experiment. At 15 minutes and 135 minutes after the onset of sepsis, the cardiac output was measured as before and the intragastric blood flow was marked as in the baseline period but using microspheres tagged with one of the markers not used earlier.

Animals in the Sepsis control group received bacteria, but no indomethacin. Animals in the indomethacin control group received indomethacin but no bacteria. The experimental group received both indomethacin and bacteria. The experimental design was otherwise the same.

The pigs were then sacrificed by anesthesia overdose. The stomachs were opened and the gastric mucosa graded by inspection for erythematous changes: 0 = normal; 1+ = minimal erythema; 2+ = maximal erythema. The stomachs were then removed and trimmed of excess fat and vessels. They were divided into antrum, corpus, and fundus. The mucosa and submucosa were stripped together from the remaining outer layer. Appropriate specimens were taken for microsphere analysis.

Microspheres were counted by the method described by McNay and Abe.[7] Radioactivity was determined using a well-type gamma scintillation spectrometer (Packard Model 5110). Each isotope was counted at its appropriate KeV setting so that only the photo peaks were counted. Background counts and crossover counts from the other isotopes were subtracted as described by Hales.[8] The actual number of microspheres recovered was then calculated from the specific activities (μCi/g) of the radioactive microspheres. Fractional gastric blood flow was then determined. Gastric blood flow was then calculated as ml/min/100 gm.

Gastric blood flow was analyzed statistically by a Wilcoxon matched pair signed rank test comparing all data to the control period. The degree of gastric mucosal erythema was analyzed by the Kruskal-Wallis One Way ANOVA to test for variation among all groups, and then by the Mann-Whitney U-test between individual groups.

Results

Independent grading of gross gastric mucosal erythema revealed a mean score of 1.5 in the *E. coli* bacteremia group, 0.14 in the indomethacin control group, and 0.5 in the indomethacin pretreated bacteremia group (analysis by the Kruskal-Wallis One Way ANOVA test indicate statistical difference ($p < 0.002$) between the three groups). Comparison of the bacteremia group to the indomethacin control and the indomethacin pretreated bacteremia groups revealed statistical difference ($p < 0.002$ and $p < 0.02$, respectively). There was no statistical significance between the indomethacin control and indomethacin pretreated bacteremia group.

The results are shown in Tables 1 to 4 and summarized in Figure 1. Core temperature rose in both groups receiving *E. coli,* but not in indomethacin controls (Table 1). There were no demonstrable changes in cardiac output in any group (Table 2). Gastric microsphere distribution (Mucosa/Full Thickness) is shown in Table 3. Gastric mucosal blood flow is shown in Table 4. *E. coli* bacteremia resulted in increased mucosal blood distribution in all parts of the stomach. Indomethacin infusion led to decreased fractional mucosal blood distribution. Bacteremic animals pretreated with indomethacin also had decreased fractional mucosal blood distribution.

TABLE 3. *Microsphere Distribution (%), (Mucosa/Full Thickness)*

	Time (min)		
	0	15	135
Escherichia coli bacteremia			
Fundus	82.5	88.0*	80.7
Corpus	81.3	92.6*	84.4
Antrum	65.3	70.0*	69.5
Indomethacin control			
Fundus	83.0	68.7*	75.9*
Corpus	86.7	66.6*	72.7
Antrum	65.2	46.0*	56.3*
Indomethacin pretreated bacteremia			
Fundus	79.8	76.7	70.7*
Corpus	82.9	76.5*	82.0
Antrum	59.8	49.7*	58.3

* Significance ($p < 0.05$).

TABLE 4. *Mean Mucosal Blood Flow* (ml/min 100 g)

	Time (min)		
	0	15	135
Escherichia coli bacteremia			
Fundus	34.4	50.6*	31.7
Corpus	22.8	34.3*	31.9
Antrum	14.7	29.5*	18.6
Indomethacin control			
Fundus	39.6	37.9	28.3
Corpus	34.8	24.5	20.8
Antrum	23.8	27.2	19.4
Indomethacin pretreated bacteremia			
Fundus	35.7	31.1	25.7
Corpus	31.1	24.5	30.0
Antrum	24.4	23.6	23.0

* Significance ($p < 0.05$).

The changes in gastric mucosal blood flow are shown in Figure 1. There were significant ($p < 0.05$) increases in gastric mucosal blood flow to the fundus (+47%), corpus (+50%), and antrum (+101%) at 15 minutes following the onset of *E. coli* infusion. These changes were not present in the indomethacin pretreated sepsis group. At 135 minutes, the increase was significant only in the antrum.

Discussion

"Stress ulceration or erosive gastritis," accompanied by gastrointestinal bleeding may occur following diverse clinical syndromes such as hypovolemic shock, trauma, multisystem failure and sepsis.[9] One suggested etiology has been that localized gastric mucosal ischemia may lead to mucosal slough.[10,11] There is experimental evidence to support this sequence of events in hemorrhagic shock.[12,13] Most stress bleeding in clinical practice, however, occurs in septic patients.

Early studies of sepsis were commonly performed using bacterial endotoxin. Using such an endotoxin model, Richardson, et al.[2] and Cheung, et al.[1] demonstrated decreased gastric blood flow. Endotoxin models are now felt to be inadequate to explain the diverse clinical syndromes of sepsis.[14] Endotoxemia leads to depression of the systemic circulation while clinical sepsis may result in a hyperdynamic state.[15] Accordingly, we elected to study gastric blood flow in a bacterial model of sepsis utilizing intravenously administered *E. coli.* This experimental approach has been utilized successfully in several prior laboratory studies of the effects of sepsis.[5,16,17] Our experimental data showed visual changes in the gastric mucosa following *E. coli* bacteremia, as previously documented by Genter[5] and Rees.[17] Intragastric blood flow data from our experiment confirmed an increase in gastric mucosal blood flow as previously reported by our group.[5] This is the opposite of the changes in gastric mucosal blood flow following endotoxemia.[1,2]

The results reported herein clearly demonstrated that pretreatment with indomethacin prevented the gastric mucosal blood flow changes associated with sepsis documented both by visual inspection and direct measurement of gastric mucosal blood flow. This was shown to be a direct gastric mucosal effect as cardiac output re-

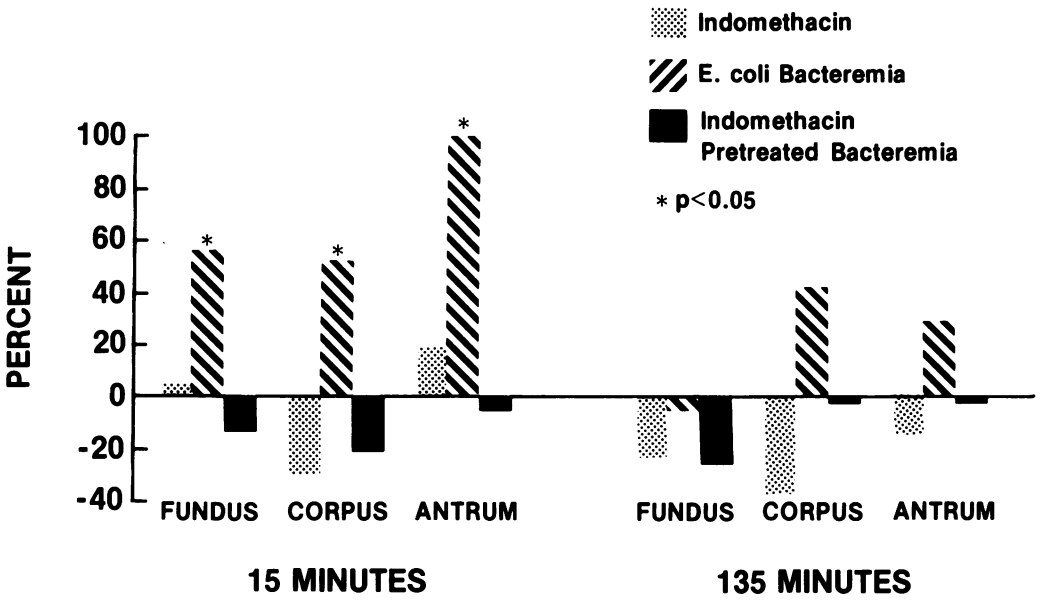

FIG. 1. Changes in gastric mucosal blood flow (%). * Significance ($p < 0.05$).

mained unchanged, despite a change in core temperature following bacteremia.

Prostaglandins are vasoactive unsaturated fatty acids produced from arachidonic acid under the influence of the enzyme cyclo-oxygenase.[18] This conversion is inhibited by nonsteroidal anti-inflammatory agents, including indomethacin and salicylates. The finding that the increased gastric mucosal blood flow associated with bacteremia could be blocked by pretreatment with indomethacin is highly suggestive that the gastric mucosal blood flow changes of sepsis are prostaglandin mediated.

Prostaglandins have been shown to be released by local tissue to the variety of stimuli that alter the basal or resting state of the animal.[18] In general, this is felt to be a homeostatic mechanism which results in a localized hyperemia. This has been documented in muscle[19] and in the kidney by our laboratory.[20] Prostaglandins have been shown to protect the gastric mucosa from various noxious stimuli including acids, alcohol, alkali, and boiling water.[21] This protective effect appears to be produced via two separate mechanisms. First, prostaglandins appear to stimulate the formation of cyclic AMP, which in turn results in increased protective gastric mucus and bicarbonate secretion. There is also a decrease in gastric acid secretion.[22] Second, prostaglandins have a vasoactive effect on gastric mucosa. This was well-documented in our study where inhibition of prostaglandin synthesis resulted in inhibition of the marked increase in gastric mucosal blood flow following bacteremia. Since synthesis of prostaglandins of all types as well as prostacycline and thromboxanes are inhibited by cyclo-oxygenase inhibitors, it is difficult to speculate which compounds are directly responsible for the phenomena documented herein. Muryobayashi et al.[23] have shown that a synthetic prostaglandin E_2 analogue may be useful in the treatment of peptic ulcer. It is difficult to speculate on the role which cyclo-oxygenase inhibitors might play, since their effect is on many vasoactive compounds which may have opposite effects. The clinical usefulness of prostaglandins and prostaglandin inhibitors in preventing stress bleeding remains to be ascertained.

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