Platelet-Activating Factor-Induced Ischemic Bowel Necrosis

An Investigation of Secondary Mediators in Its Pathogenesis

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The authors have previously reported a model of ischemic bowel necrosis produced in the rat by synthetic platelet-activating factor (PAF) or a combination of PAF and bacterial endotoxin. Because rat platelets are refractory to PAF and thromboemboli were not found in the mesenteric or intestinal microvasculature, they suspected that secondary mediators were involved in the pathogenesis of bowel necrosis. They have found the following 1) lipoxygenase products of arachidonic acid, especially leukotrienes (LT), probably played an important role in the pathogenesis of bowel necrosis, because diethylcarbamazine (an inhibitor for LTA synthesis) and FPL55712 (LT antagonist) ameliorated, and at times completely prevented, the lesions. NDGA (a nonspecific lipoxygenase inhibitor) was less effective, probably because of its additional effect on cyclooxygenase inhibition. 2) Verapamil, a calcium channel blocker, ameliorated the disease. 3) Thromboxane A_2, a potent vasoconstrictor, was probably not responsible for the ischemia of the gastrointestinal tract. This is suggested by the ineffectiveness of OKY-046 in preventing bowel necrosis. 4) Prostaglandin (PG) E_1 infusion often prevented the bowel necrosis, which suggested beneficial effects of vasodilating PGs, probably released as a defense mechanisms. 5) Indomethacin aggravated the disease, probably by inhibiting PG release and shifting the metabolic pathway toward the lipoxygenase pathway. 6) Antihistamine and antiserotonin had no effect, which suggested that these mediators were not involved in the pathogenesis of bowel necrosis. 7) Shock produced by PAF was probably not the only cause of bowel necrosis, because reversal of the hypotension did not always prevent the development of bowel necrosis. 8) Hemoconcentration (increased vasopermeability) and leukopenia induced by PAF did not correlate with the development or severity of bowel necrosis. (Am J Pathol 1986, 122:231–239)

ISCHEMIC bowel necrosis is an often lethal condition that may occur in patients of all ages. Whereas its cause may sometimes be traced to well defined thromboembolic occlusion of large mesenteric vessels, in most cases the necrosis supervenes without apparent intravascular thrombi. The etiology is probably multifactorial, and the pathogenesis is complex. We have successfully produced a model of ischemic bowel necrosis in rats by administrating synthetic platelet-activating factor (PAF), (also termed AGEPC[1] or PAF-acether[2]) or a combination of PAF and bacterial endotoxin.[3] This model is of interest because PAF is a naturally occurring substance synthesized by many cells,[4–6] and the lesions obtained were morphologically identical to that of human patients with necrotizing enterocolitis.[3] The mechanism of action of these substances and the pathogenesis of bowel necrosis remain unknown, but these effects were shown to be independent of platelet action.[3] This is because rat platelets are refractory to the effects of PAF on platelet aggregation[3,7] and secretion.[7] Since thromboemboli were not the cause of ischemia and subsequent necrosis, we considered it possible that certain secondary mediators with potent vascular effects were released in our model. Among the naturally occurring vasoactive agents, prostaglandins (PG) and other arachidonic acid metabolites seemed likely candidates. PGs are secreted by both mesenteric vessels and the gastrointestinal tract. Rat jejunal tissue *in vitro* synthesized large amounts of PGD_2 and small amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$.[8] $PGF_{2\alpha}$ and thromboxane (Tx) have also been demonstrated.[9–12] Leukotriene (LT) C_4 and D_4 have been shown to constrict canine mesenteric

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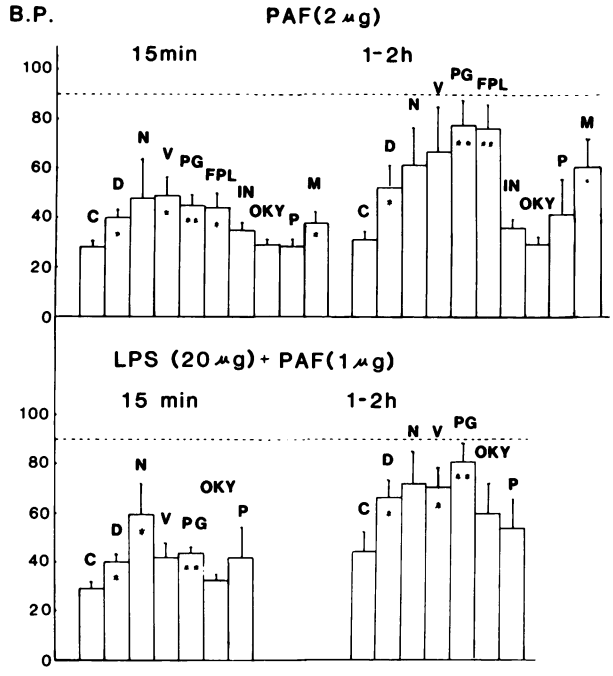

Figure 1—Effects of various drugs on PAF-induced bowel necrosis. Changes in mean systemic blood pressure. The inducing agents used was either PAF (2 μg) alone (**upper panel**) or LPS (20 μg) + PAF (1 μg) (**lower panel**). The **left panel** shows initial change in blood pressure, the **right panel** shows the sustained blood pressure change represented by the medial value at 1–2 hours. The following abbreviations are used: *C*, control; only inducing agents were given. Other groups also received inducing agents(s), but were pretreated with diethylcarbamazine (*D*), 350 mg/kg; NDGA (*N*), 2 mg/kg; Verapamil (*v*), 0.5 mg/kg; PGE_1 (*PG*), 2 mg/kg/min; FPL55712 (*FPL*), 5 mg/kg; indomethacin (*IN*), 5 mg/kg; OKY-046 (OKY), 0.5 mg/kg; pyralamine (*P*), 0.5 mg/kg; methysergide (*M*), 0.5 mg/kg. The lower limit of normal range is shown as the *dotted line*. Mean ± SEM. * $P \leq 0.05$. ** $P \leq 0.01$.

vasculature[13,14] and release of LTC_4-like immunoreactive material has been shown to occur in the anaphylactic guinea pig vascular bed.[15] In addition, abundant mast cells, eosinophils, and macrophages reside in the intestinal wall, all of which are rich sources of LTs.[16–19] The present study suggests that 1) at least an important portion of PAF-induced bowel injury was mediated through the action of LTs; 2) Tx was not significantly involved in the pathogenesis of this model; and 3) vasodilating PGs were beneficial to the host and could counteract the vasoconstricting effect of LTs.

Materials and Methods

Platelet-activating factor, L-α-lecithin, β-acetyl-, γ-o-alkyl, was purchased from CalBiochem-Behring, La Jolla, California. Stock solution in ethanol was kept at −70 C. Working solution (in 2.5 mg/ml bovine serum albumin–saline solution) was prepared fresh daily. Lipopolysaccharide (LPS) from *Salmonella typhosa* was purchased from Difco Laboratories, Detroit, Michigan. Fresh saline solution was prepared before use. PGE_1, nordihydroguaiaretic acid (NDGA), and diethylcarbamazine were purchased from Sigma Chemical Co., St. Louis, Missouri. OKY-046 was a generous gift from ONO Pharmaceutical Co., Osaka, Japan. FPL55712 was generously provided by Sandoz Research Institute, East Hanover, New Jersey. PGE_1 stock solution (10 mg/ml ethanol) was kept at −20 C. All other drugs were trade drugs purchased from a local pharmacy; solutions were made before use.

Male Sprague–Dawley rats with body weight of 280 ± 20 g were used in all experiments. The rats were anesthetized with Nembutal; the carotid artery was cannulated and connected to a blood pressure transducer (Harvard Apparatus) and a recorder. The jugular vein was cannulated for injection or infusion of drugs. The abdomen was incised along the midline, and 2 μg PAF (approximately 7 μg/kg), or 20 μg LPS immediately followed by 1 μg PAF were injected into the abdominal aorta at the level of the renal arteries as previously described.[3] The abdominal incision was covered with gauze moistened with warm saline, and the intestines were exposed and examined periodically under a stereomicroscope. Blood samples were drawn at time 0, 30, 60, and 120 minutes for determination of hematocrit (Hct) and WBC count. In some experiments, when the intestinal lesion was grossly visible (15–60 minutes), a transmural biopsy of the intestinal wall was taken before the time of sacrifice (2 hours). Immediately before sacrifice, 5 ml of Evans blue (2 g/dl) was injected into the jugular vein to assess the degree of perfusion. Blocks of small intestinal tissue were taken from the poorly perfused or grossly diseased areas. (The large intestine was usually spared.) If there were no gross lesions or poorly perfused areas visible, 20–30 random blocks were taken from the small intestine for morphologic examination. A complete postmortem examination was also performed. If poorly perfused areas were observed in other organs, sections were taken for microscopic examination. Routine hemotoxylin and eosin (H & E) staining of paraffin-embedded tissues was done for determination of the microscopic changes of the intestine and other organs.

All medications except PGE_1 and methysergide were injected slowly into the jugular vein 10 minutes before administration of PAF. Continuous PGE_1 infusion into the jugular vein was begun 15–20 minutes before PAF injection; methysergide was given by mouth with a syringe with an animal feeding needle 45 minutes before PAF injection.

The values for blood pressure, Hct, WBC, and perfusion percentage for each control and treatment group were subjected to null hypothesis testing (for small samples where df <31) using the Student *t* test. With a com-

puter program, SPSS-PC (SPSS Inc., Chicago, Ill, 1985) the F distribution on the means of the two populations (control versus treated) were compared and found different; thus the *t* test with separate, rather than pooled, variance estimates was used. This analysis yielded the means and standard errors of the mean (SEM) for each paired sample. If the two-tailed probability of the *t* test was greater than 0.05, the null hypothesis (control and treated groups values are not different) was accepted; if equal or less than 0.05, the null hypothesis was rejected. Results are reported as significant if *P* was 0.05 or less.

Results

There was considerable individual variation of response to the drugs administered. This variability from animal to animal is shown in the tables included as the Appendix. Figures in the text express mean values. There was generally a correlation between the extent of gross and microscopic disease. When gross abnormality was noted, microscopic lesions were always present. However, mild microscopic lesions often escaped gross inspection and examination with the aid of steromicroscope. In other words, a grossly normal bowel with 100% perfusion could contain mild microscopic lesions. Thus, the presence or absence of bowel necrosis was documented by histologic examination in all experiments. The light-microscopic findings are also shown in the tables in the Appendix.

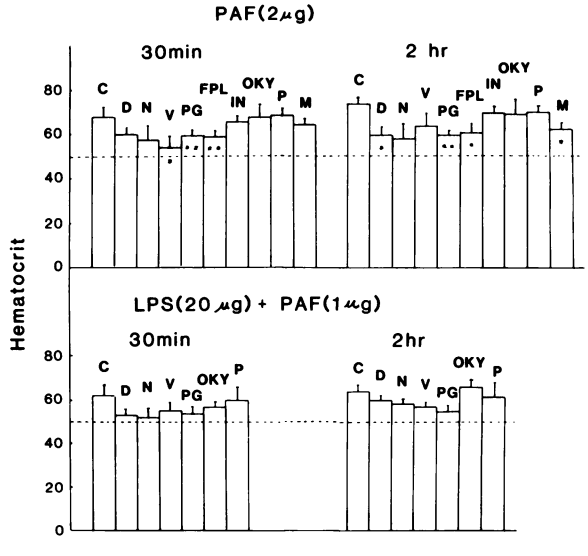

Figure 2—Effects of drugs on PAF-induced bowel necrosis. Changes in hematocrit (see Figure 1 legend for abbreviations and symbols). The upper limit of normal Hct is shown as the *dotted line*.

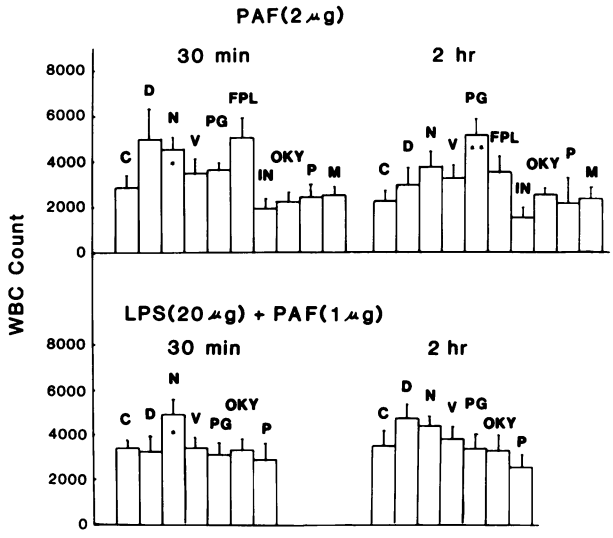

Figure 3—Effects of drugs on PAF-induced bowel necrosis. Changes in peripheral WBC count (see Figure 1 legend for abbreviation and symbols). The WBC count before PAF administration in 50 rats was 6875 ± 230.

Effects of LPS and PAF on the Gastrointestinal System, Systemic Effects, eg, Blood Pressure, Hct, WBC Count, and Effects on Other Organs

Injection of 2 μg PAF or combined administration of a lower dose of PAF (1 μg) and LPS (20 μg) invariably resulted in necrosis of the small bowel (Appendix, Table 2). The majority of the animals developed moderate to severe necrotic gross lesions, and variable microscopic lesions ranging from loss of superficial lining of villi (mild) to sloughing off of villi (moderate) and, rarely, muscular necrosis (severe). Although the changes of hypotension, hemoconcentration, leukopenia, and intestinal hypoperfusion were not significantly different between these two groups (Figures 1–4), the extent of the microscopic lesions was different. In the group receiving 2 μg of PAF, all showed moderate or severe lesions, whereas 2 of 11 rats treated with the lower dose of PAF showed mild lesions (Table 2, Appendix). No thrombi or inflammatory cell infiltrates were observed in the intestinal wall or in the mesenteric vessels. Other organs showed no abnormalities, except for PMN sequestration in the pulmonary vessels. An immediate severe drop of blood pressure invariably followed PAF administration (Figure 1). Hypotension often became less marked after 30 minutes (especially in those animals receiving the smaller dose of PAF (1 μg) and stabilized in 1–2 hours. Less than 10% of the animals receiving a higher dose (2 μg) of PAF died of severe sustained shock. There was always an increase of Hct (Figure 2) and a decrease of peripheral WBC count (Figure 3), which was sustained for 1–2 hours. The platelet count

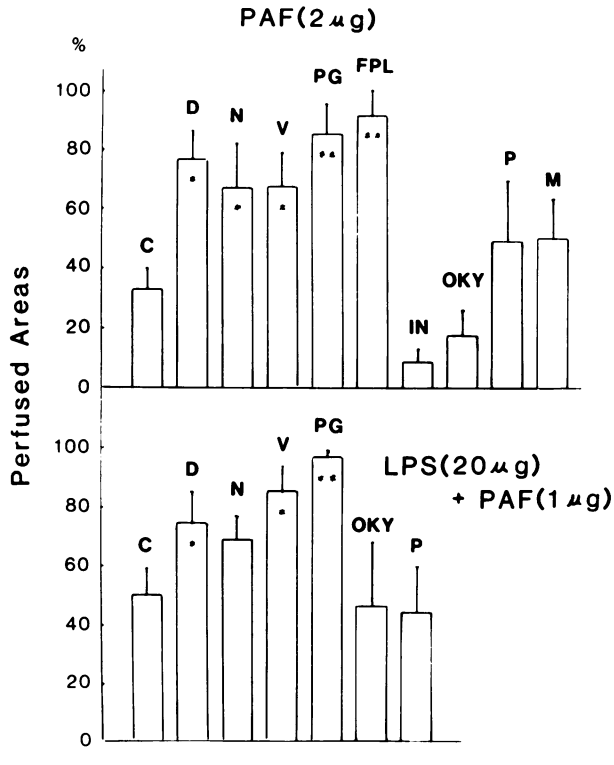

Figure 4—Effects of drugs on PAF-induced bowel necrosis. Perfused areas as a percentage of the total length of the small intestine, determined by Evans blue injection at 2 hours (see Figure 1 legend for abbreviations). (Notice that most of the rats pretreated with indomethacin died before 2 hours, and Evans blue was injected after death. Thus the values may not represent the true perfusion extent.)

did not show any important changes, as reported previously.[3]

Observation of the intestinal perfusion showed that vasoconstriction of the mesenteric vessels took place almost immediately after injection of PAF. However, the time of development of discoloration of the bowel varied from 15 minutes to 1 hour. When Evans blue was injected at the end of the experiment, the hypoperfused areas generally correlated with the grossly discolored areas. Thus, only data on perfusion are presented (Figure 4). LPS alone, at the usual dose administered, exhibited no effects on blood pressure, Hct, or WBC counts. When the LPS dose was doubled (40 μg), a mild hypotensive effect was observed. However, no hypoperfusion of the bowel, necrosis, increased Hct, or leukopenia were seen.

Effects of Pretreatment With Lipoxygenase Inhibitors, Diethylcarbamazine, FPL 55712, and NDGA

Three lipoxygenase inhibitors of different mechanism of action were used. NDGA (2 mg/kg), a nonspecific inhibitor of lipoxygenase (and, at high dose, also an inhibitor of cyclooxygenase), diethylcarbamazine (350 mg/kg), reported to be an inhibitor of LTA synthesis,[20] or FPL55712 (5 mg/kg), an antagonist for LT receptors, were injected into the jugular vein before administration of PAF and LPS. The results are shown in Figures 1–4 (also see Appendix, Table 3 and 4). Among these three drugs, FPL55712 was the most effective in lessening the severity of the lesions (Figure 4). It prevented the gross necrotic lesions in 6 of 7 animals and showed none or only mild microscopic lesions in all except one (Table 3). Diethylcarbamazine significantly improved the hypotension and intestinal perfusion (Figures 1 and 4) and decreased the necrotic changes, entirely preventing, in some instances (4 out of 14 rats treated, Table 3), the development of necrosis. However, diethylcarbamazine was less effective in prevention of increased vascular permeability and had no effect on leukopenia (increased Hct) (Figures 2 and 3). NDGA was the least effective of the three drugs. However, in a single animal (see Appendix, Table 4, Rat 1), it completely prevented the development of bowel necrosis. Interestingly, there were several instances (see Appendix, Table 4, Rats 8 and 9) when NDGA corrected the hypotension but failed to prevent the necrosis of the bowel, indicating that the pathogenesis of bowel necrosis was not due to the hypotensive action of PAF and LPS. Nonetheless, when severe sustained hypotension occurred, it was invariably accompanied by bowel necrosis.

Effects of Pretreatment With Verapamil

Verapamil (0.5 mg/kg), a calcium channel blocker, significantly ameliorated the disease (Figures 1–4) (see Appendix, Table 5). The bowel appeared well perfused and grossly unaffected in 7 of 14 rats treated. However, by careful microscopic examination, necrotic lesions of mild to moderate degree could still be found in some sections (4 of 7). Verapamil lessened the initial increase in permeability caused by PAF (Figure 2) but had no effect on prolonged leukopenia (Figure 3).

Effects of Prostaglandin E_1 and Inhibitors of PGs and TX

Continuous infusion of PGE_1 intravenously (2 mg/kg/min) significantly improved the intestinal perfusion (Figure 4) and prevented gross bowel lesions in 11 of 14 animals treated. Three of these 11 rats still showed mild microscopic lesions (see Appendix, Table 6). PGE_1 significantly corrected the hypotension and hemoconcentration caused by PAF but showed no effect on WBC count (Figures 1–3), which indicated that the

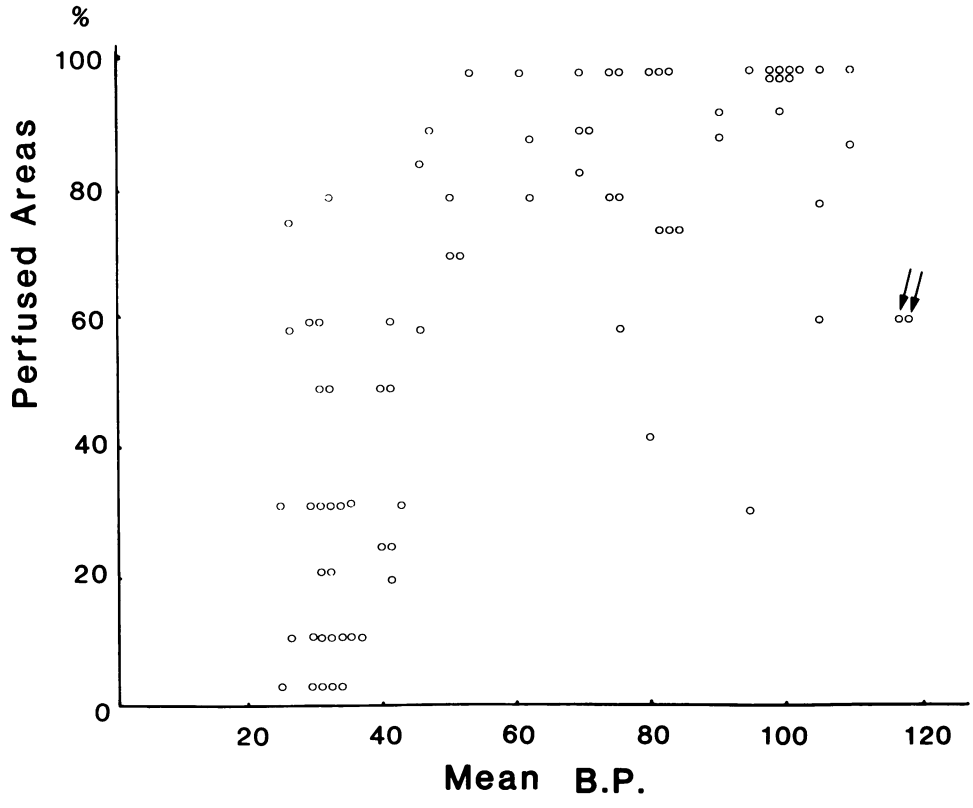

Figure 5—Relationship between final blood pressure (at 2 hours) and intestinal perfusion (percentage of total length) in all experiments. The *arrows* indicate 2 animals pretreated with NDGA that developed bowel necrosis despite the complete correction of hypotension.

pathogenesis of bowel necrosis is independent of an effect of PAF on leukopenia. Indomethacin (5 mg/kg), in contrast, aggravated the condition (Figures 1–4) and resulted in death within 2 hours in 5 of 6 animals treated. Postmortem examination revealed poor perfusion of the heart and lungs in addition to the poor perfusion of the gastrointestinal system and massive bowel necrosis. OKY-046 (0.5 mg/kg), a Tx inhibitor, showed no beneficial effects on the development of bowel necrosis in all 8 animals treated (4 in each group) (Figures 1–4). In addition, two animals were pretreated with 5 mg/kg of OKY-046 before administration of PAF. Although this dose was not toxic by itself, pretreatment with this dose aggravated the disease. Both animals died within 45 minutes with poor perfusion of bowel.

Antihistamine and Antiserotonine

Pyralamine (0.5 mg/kg intravenously) showed no effect in preventing the development of bowel necrosis caused by PAF (n=5) or PAF with LPS (n=4) in the 9 rats treated (Figures 1–4). Methysergide (0.5 mg/kg) improved intestinal perfusion in only 1 of 6 animals treated. The overall effect of this drug was not statistically significant (Figures 1–4).

Discussion

The systemic effects of PAF in the rat include profound and sustained hypotension (shock), hemoconcentration due to increased vascular permeability, and leukopenia probably secondary to polymorphonuclear leukocyte (PMN) sequestration in the pulmonary vessels. The pathogenesis of bowel necrosis seems to be independent of PAF-induced hemoconcentration and leukopenia. This conclusion is justified by the fact that when some of the medications (eg, Verapamil, PGE_1, and FPL55712) prevented the development of bowel necrosis, Hct and WBC counts remained similar to those in animals without pretreatment. There was generally a good correlation between prolonged, severe hypotension (blood pressure $\leqslant 40$) and development of bowel necrosis (Figure 5). In other words, when the former was observed, the latter invariably occurred, but shock was not the only factor that caused bowel necrosis. In some instances, when NDGA pretreatment reversed the hypotension, bowel necrosis still developed (Figure 5, arrows) (Appendix, Table 3). These results indicate that the pathogenesis of bowel necrosis is probably more complex than could be accounted for by simple hypotension. Various secondary mediators with local vasoactive effects were probably released.

Our studies suggest an important role of lipoxygenase products or arachidonic acid, especially leukotrienes, in the pathogenesis of PAF-induced bowel necrosis. This conclusion is based on the following observations: 1) Diethylcarbamazine, a LTA synthetase inhibitor,[18] interfered with the development of the disease. 2) FPL55712, an antagonist for LT receptors, significantly improved the development of intestinal necrosis. 3) 5-Lipoxygenase is calcium-dependent,[21] and Verapamil, a Ca^{2+} channel blocker (which inhibits Ca^{2+} influx required for lipoxygenase activation) lessened the necrotic lesions. (The mechanism action of Verapamil is not clear-cut. It also inhibits calcium influx required for muscle contraction.) 4) In some instances NDGA, a nonspecific lipoxygenase inhibitor, was capable of ameliorating the necrosis. However, NDGA was not as effective as the other three drugs, probably because it also inhibited cyclooxygenase products, including PGE and prostacyclin (a potent vasodilator), which, in turn, may have aggravated the vasoconstriction of the mesenteric vascular bed. 5) Indomethacin, a potent cyclooxygeanse inhibitor, aggravated the disease. This was probably due to *a*) decreased production of the abovementioned vasodilating PGs and *b*) a shift of the reaction from the cyclooxygenase pathway to the lipoxygenase pathway, which thus further increased LT production. Preliminary experiments showed that injection of LTC_4 (5 μg/kg) into the mesenteric vascular bed resulted in necrosis of the small bowel.

LTs are naturally occurring substances secreted by many inflammatory cells, such as PMNs,[22] macrophages,[19] eosinophils,[18] mast cells,[16,17] and possibly basophils. Although no PMN infiltrates were seen in the bowel wall, mast cells, eosinophils, basophils, and macrophages are normal residents in the intestinal walls and in the connective tissue of the mesenteric vascular bed. It is possible that one or more types of these inflammatory cells were involved in the cellular mechanism of PAF-induced bowel necrosis.

The involvement of LTs as secondary mediators of PAF was not surprising. Although there has been no study showing that PAF causes PG and LT release in the GI tract, it has been suggested that arachidonic acid metabolites (PGs, LTs) are secondary mediators for PAF-induced bronchoconstriction.[23,24] Furthermore, PAF causes release of arachidonic acid in platelets,[23] stimulates arachidonic acid metabolism in PMNs,[25] and results in LT production in the lungs.[24]

It is of interest that injection of PAF into the abdominal aorta resulted in bowel necrosis only, but not renal necrosis. It has been reported that injection of LTs C_4, D_4, or E_4 into the canine mesenteric vascular beds resulted in reduction of blood flow,[13,14] whereas injection of the same LTs into the renal artery caused little or no changes in renal blood flow[14] or even an increase of renal blood flow.[13] Similar differential effects of LTs may exist in the rat as well, thereby accounting for isolated involvement of gastrointestinal tract, to the exclusion of other organs.

Our data further suggest that vasodilating PGs were probably also secreted in response to PAF, presumably as a defense mechanism. Indomethacin aggravated the disease, whereas PGE_1 infusion ameliorated it. Thromboxane A_2, potent vasoconstrictor and also a metabolite of the cyclooxygenase pathway, was probably not involved in the mediation of bowel necrosis.

LPS itself was not the main agent that caused bowel necrosis. We propose that it only augmented the development of this lesion because LPS by itself exhibited only mild hypotensive action, had no effect on Hct or WBC count, and caused no necrosis or hypoperfusion of the intestine. However, bacteria and their toxins have long been suspected of playing an important role in the pathogenesis of ischemic bowel necrosis, especially neonatal necrotizing enterocolitis[26,27] and pigbel.[28] The fact that a combination of LPS and PAF could produce the same effect as half the dose of PAF suggests that the pathogenesis of ischemic bowel necrosis could be multifactorial and requires the cooperation of several extrinsic and intrinsic factors, including inflammatory mediators and bacterial products.

References

1. Pinckard RN, McManus LM, Halonen M, and Hanahan DJ: Acetyl glyceryl ether phosphorycholine: platelet-activating factor. Int Arch Allergy Appl Immunol 1981, 66(Suppl 1):127–136
2. Vargaftig BB, Chignard M, Benveniste J, Lefort J, Wal F: Background and present status of research on platelet-activating factor (PAF-acether). Ann NY Acad Sci 1981, 370:119–137
3. Gonzalez-Crussi F, Hsueh W: Experimental model of ischemic bowel necrosis. The role of platelet activating factor and endotoxin. Am J Pathol 1983, 112:127–135
4. Camussi G, Aglietta M, Coda R, Bussolino F, Piacibello W, Tetta C: Release of platelet-activating factor (PAF) and histamine. II. The cellular origin of human PAF: monocytes, polymorphonuclear neutrophils and basophils. Immunology 1981, 42:191–199
5. Mencia-Huerta JM and Benveniste J: Platelet-activating factor (PAF-acether) and macrophages: II. Phagocytosis associated release of PAF-acether from rat peritoneal macrophages. Cell Immunol 1981, 57:281–292
6. Camussi G, Aglietta M, Malavasi F, Tetta C, Piacibello W, Sanavio F, Bussolino F: The release of platelet activating factor from human endothelial cells in culture. J Immunol 1983, 131:2397–2402
7. Sanchez-Crespo M, Alonso F, Iñarrea P, Egido J: Nonplatelet-mediated vascular actions of 1-0-alkyl-2-acetyl-sn-3-glyceryl-phosphorylcholine (a synthetic PAF), Pharmacologie de l'inflammation et l'allergie: Lipids et cellules. Edited by F Russo-Marie, B Vargaftig, J Beneveniste. Paris, Editions INSERM, 1981, pp 473–478
8. Peskar BM, Weiler H, Kröner EE, Peskar BA: Release of prostaglandins by small intestinal tissue of man and

rat in vitro and the effect of endotoxin in the rat in vivo. Prostaglandins 1981, 21 (suppl):9-14
9. LeDuc LE, Needleman P: Regional localization of prostacycline and thromboxane synthesis in the dog stomach and intestinal tract. J Pharmacol Exp Ther 1979, 211:181-188
10. Peskar BM, Günter B, Peskar BA: Prostaglandins and prostaglandin metabolites in human gastric juice. Prostaglandins 1980, 20:419-427
11. Walker R, Wilson KA: Gastroenterology, prostaglandins, bradykinin and rat ileum. Advances in Prostaglandin and Thromboxane Research. Vol 8. Edited by B Samuelsson, PW Ramwall and R Paoletti. New York, Raven Press, 1980, pp 1573-1575
12. Smith GS, Warhurst G, Turnberg LA: Synthesis and degradation of prostaglandin E_2 in the epithelial and subepithelial layers of the rat intestine. Biochim Biophys Acta 1982, 713:684-687
13. Feigen LP: Differential effects of leukotrienes C_4, D_4 and E_4 in the canine renal and mesenteric vascular beds. J Pharmacol Exp Ther 1983, 225:682-687
14. Chapnick BM: Divergent influences of leukotrienes C_4, D_4 and E_4 on mesenteric and renal blood flow. Am J Physiol 1984, 246:H518-H524
15. Dembinska-Kiec A, Simmet T, Peskar BA: Release and vasoconstrictor effect of leukotriene C_4-like immunoreactive material in the guinea pig mesenteric vascular bed. Eur J Pharmacol 1984, 101:259-262
16. MacGlashan DW, Jr, Schleimer RP, Peters SP, Schulman ES, Adams GK, III, Newball HH, Lichtenstein LM: Generation of leukotrienes by purified human lung mast cells. J Clin Invest 1982, 70:747-751
17. Razin E, Mencia-Huerta JM, Lewis RA, Corey EJ, Austen KF: Generation of leukotriene C_4 from a subclass of mast cells differentiated in vitro from mouse bone marrow. Proc Natl Acad Sci 1982, 79:4665-4667
18. Weller PF, Lee CW, Foster DW, Corey EJ, Austen KF, Lewis RA: Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: Predominant production of leukotriene C_4. Proc Natl Acad Sci USA 1983, 80:7626-7630
19. Rouzer CA, Scott WH, Cohn ZA, Manning JM: Mouse peritoneal macrophages release leukotriene C in response to a phagocytic stimulus. Proc Natl Acad Sci USA 1980, 77:4928-4932, 1980
20. Mathews WR, Murphy RC: Inhibition of leukotriene synthesis in mastocytoma cells by diethylcarbamazine. Biochem Pharmacol 1982, 31:2129-2132
21. Feinstein MB, Shaafi RI: Role of calcium in arachidonic acid metabolism and in the actions of arachidonic acid-derived metabolites. Calcium and Cell Function, Vol 4. Edited by WY Cheung. New York, Academic Press, 1983, pp 337-376
22. Borgeat P, Samuelsson B: Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. J Biol Chem 1979, 254:2643-2646
23. Vargaftig BB, Lefort H, Chignard M, Benveniste J: Platelet-activating factor induces a platelet-dependent bronchoconstriction due to the formation of prostaglandin derivatives. Eur J Pharmacol 1980, 65:185-192
24. Velkel NF, Worthen S, Reeves JT, Henson PM, Murphy RC: Nonimmunological production of leukotrienes induced by platelet activating factor. Science 1982, 218:286-288
25. Chilton FH, O'Flaherty JT, Walsh CE, Thomas MJ, Wykle RL, DeChatelet LR, Waite BM: Platelet activating factor stimulation of the lipoxygenase pathway in polymorphonuclear leukocytes by 1-0-alkyl-2-0-acetyl-sn-glycero-3-phosphocholine. J Biol Chem 1982, 257: 5402-5407
26. Cashore WJ, Peter G, Lauermann M, Stonestreet BS, Oh W: Clostridia colonization and clostridial toxin in neonatal necrotizing enterocolitis. J Pediatr 1981, 98:308-311
27. Kliegman RM: Neonatal necrotizing enterocolitis: Implications for an infectious disease. Pediatr Clin North Am 1979, 26:327-344
28. Lawrence G, Walker PD: Pathogenesis of enteritis necroticans in Papua New Guinea. Lancet 1976, 1:125-126

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Appendix

To simplify the presentation of the data, arbitrary criteria were used for estimating the degree of damage, as defined in Table 1.

Table 1—Criteria Used to Present Data in the Following Tables

	0	+	+ +	+ + +
Hypotension	>90	60–90	40–60	<40
Hct	<51	51–55	56–59	>59
Decrease in WBC count	0	1000–2000	2000–3000	>3000
Hypoperfusion*	None	5 cm	5–30 cm	>30 cm
Gross lesion†	None	5 cm	5–30 cm	>30 cm
Microscopic lesion‡	Normal	Loss of epithelial cells	Destruction of villi (mucosal necrosis)	Transmural necrosis

* Extent of hypoperfusion was determined by Evans blue injection.
† Gross lesion was decided by the length of discolored bowel before the injection of Evans blue.
‡ Microscopic changes of the most severely involved area of all blocks taken are presented in the following tables.

Table 2—Effects of PAF (2 μg) Alone or LPS (20 μg) Plus PAF (1 μg)

	Inducing Agent		Hypotension				Intestinal necrosis						
							15 minutes		1 hour		2 hours		
Rat	LPS (μg)	PAF (μg)	15 minutes	1–2 hours	Hct (2 hours)	WBC (2 hours)	Gross	Micro	Gross	Micro	Hypo-perf	Gross	Micro
1	0	2	+ + +	+ + +			+ + +		+ + +	+ +	+ + +	+ + +	+ +
2	0	2	+ + +	+ + +			+ + +		+ + +		+ + +	+ + +	+ +
3	0	2	+ + +	+ + +	+ + +	+ + +	+ +		+ + +		+ + +	+ + +	+ +
4	0	2	+ + +	+ + +	+ + +	+ + +	+ + +		+ + +		+ + +	+ + +	+ + +
5	0	2	+ + +	+ + +	+ + +	+ + +	+ +		+ +		+ +	+ +	+ +
6	0	2	+ + +	+ + +	+ + +	+ + +	+ + +		+ + +		+ + +	+ + +	+ +
7	0	2	+ + +	+ + +	+ + +	+ + +	+ + +		+ + +		+ + +	+ + +	+ +
8	20	1	+ + +	+ +			+		+ +		+ +	+ +	+ +
9	20	1	+ + +	+			0	0	+		+ +	+ +	+ +
10	20	1	+ +	+ +	+ +	+ +	0	0	+	+	+	+	+
11	20	1	+ +	+			0	0	+		+ +	+ +	+ +
12	20	1	+ +	+ +			0	0	0	+	+	+	+
13	20	1	+ + +	+ +	+ +	+ +	0	+	+ + +	+ +	+ + +	+ + +	+ +
14	20	1	+ +	+ +			+	+	+ +	+ +	+ +	+ +	+ +
15	20	1	+ +	+ + +	+ + +	+ + +	+ +		+ + +		+ + +	+ + +	+ + +
16	20	1	+ +	+ +			+ + +		+ + +		+ + +	+ + +	+ + +
17	20	1	+ + +	+ + +	+ + +	+ +	+ +		+ + +		+ + +	+ + +	+ + +
18	20	1	+ +	+ + +	+ + +	+ +	+ +		+ +		+ +	+ +	+ +

Table 3—Effects of Diethylcarbamazine (Rats 1–14) and FPL 55714 (rats 15–21) on Experimental Bowel Necrosis

	Inducing agent		Hypotension				Intestinal necrosis						
							15 minutes		1 hour		2 hours		
Rat	LPS (μg)	PAF (μg)	15 minutes	1–2 hours	Hct (2 hours)	WBC (2 hours)	Gross	Micro	Gross	Micro	Hypo-perf	Gross	Micro
1	0	2	+ +	0			0		0		0	0	+
2	0	2	+ + +	0	+	+ +	0		0		0	0	0
3	0	2	+ +	+ +	+	+ +	0		+		+ +	+ +	+ +
4	0	2	+ +	+ + +	+ + +	+ +	+		+ + +		+ + +	+ + +	+ +
5	0	2	+ +	+ +	+ + +	+ +	+		+ +	+ +	+ +	+ +	+ +
6	0	2	+ +	+ +	+ +	+ +	+		+		+	+	+ +
7	20	1	+ +	+	0	+ +	0		0		0	0	0
8	20	1	+ + +	+ + +			+ +		+ +	+ +	+ +	+ +	+ +
9	20	1	+ +	+ +	+ +	+ +	0	0	0	+	0	0	0
10	20	1	+ +	+	+ +	+ +	+ +		+ +		+ +	+	+ +
11	20	1	+ +	+ +	+ +	+ +	+ +		+ +		+ +	+	+ +
12	20	1	+ +	0	+ +	+ +	+		+		+	+	0
13	20	1	+ + +	+ +	+	+ +	+		+		+	+	+ +
14	20	1	+ +	+	+ + +	+ +	+		+		+	+	+ +
15	0	2	+ +	+	+ +	+	0		0		0	0	0
16	0	2	+ +	+	+ + +	+ +	0		0		0	0	+
17	0	2	+ + +	+ + +	+ + +	+ + +	+ +		+ +		+ +	+ +	+ +
18	0	2	+ +	0	+ +	+ +	0		0		0	0	0
19	0	2	+ +	0	+	+ +	0		0		0	0	0
20	0	2	+ +	0	+	+ +	0		0		0	0	+
21	0	2	+ +	0	+	+ +	0		0		0	0	+

Table 4—Effects of NDGA on Experimental Bowel Necrosis

	Inducing agent		Hypotension				Intestinal necrosis						
							15 minutes		1 hour		2 hours		
Rat	LPS (μg)	PAF (μg)	15 minutes	1–2 hours	Hct (2 hours)	WBC (2 hours)	Gross	Micro	Gross	Micro	Hypo-perf	Gross	Micro
1	0	2	+	0	0	+ +	0		0		0	0	0
2	0	2	+ +	+	+	+ +	0		0		0	+	+ +
3	0	2	+ + +	+ + +	+ +	+ + +	+ +		+ + +		+ + +	+ + +	+ +
4	0	2	+ +	+ +	+ +	+ + +	+		+ +		+ + +	+ + +	+ +
5	0	2	+ + +	0	+ + +	+ + +	0		+		+	+	+
6	0	2	+ +	+ +	+ + +	+ + +	+ +		+ +		+ +	+ +	+ +
7	20	1	0	+ +	0	+ +	0	0	0	+	0	0	+
8	20	1	0	0	0	+ +	0	0	+		+ +	+ +	+ +
9	20	1	+	0			0	0	0	0	+	+	+
10	20	1	+ +	+ +	+ +	+ +	0	+	+	+	+	+	+
11	20	1	+ +	+ +			0	0	+	+ +	+	+	+ +
12	20	1	+ +	+ +	+ +	+ +	0		+		+	+	+
13	20	1	+ +	+	+ + +	+ +	+		+		+	+	+ +

Table 5—Effects of Verapamil on Experimental Bowel Necrosis

	Inducing agent		Hypotension				Intestinal necrosis						
							15 minutes		1 hour		2 hours		
Rat	LPS (μg)	PAF (μg)	15 minutes	1–2 hours	Hct (2 hours)	WBC (2 hours)	Gross	Micro	Gross	Micro	Hypo-perf	Gross	Micro
1	0	2	+ +	0			0		0		0	0	+
2	0	2	+ +	0			0		0		0	0	0
3	0	2	+ +	0	0	+ + +	0		0		0	0	0
4	0	2	+ + +	+ + +	+ +	+ + +	+ +		+ + +		+ + +	+ + +	+ +
5	0	2	+ + +	+ +	+ +	+ + +	+ +		+ +		+ +	+ +	+ +
6	0	2	+ +	+ + +	+ + +	+ + +	+ +		+ +		+ + +	+ + +	+ +
7	20	1	+ + +	+ +			+	+	+ +	+ +	+ +	+ +	+ +
8	20	1	+ +	+	0	+ +	0	0	+		+	+	+
9	20	1	+ + +	+ + +	+	+ + +	0	0	+		0	0	0
10	20	1	+ +	+	0	+ + +	0		0		0	0	+
11	20	1	+ + +	+ +	+	+ +	0	0	0	0	0	0	+
12	20	1	+ +	+			0		0	0	0	0	0
13	20	1	+ + +	+ +	+ + +	+ + +	+		+ +		+ +	+ +	+ +
14	20	1	+ +	+	+	+ +	+		+ +		+ + +	+ + +	+ +

Table 6—Effects of PGE_1 on Experimental Bowel Necrosis

	Inducing agent		Hypotension				Intestinal necrosis						
							15 minutes		1 hour		2 hours		
Rat	LPS (μg)	PAF (μg)	15 minutes	1–2 hours	Hct (2 hours)	WBC (2 hours)	Gross	Micro	Gross	Micro	Hypo-perf	Gross	Micro
1	0	2	+	0	+	+	0		0		0	0	0
2	0	2	+ +	+	+ + +	0	0		0		0	0	0
3	0	2	+ + +	+	+ + +	0	0		0		0	0	0
4	0	2	+ + +	+ + +	+ + +	+ + +	+ +		+ + +	+ +	+ + +	+ + +	+ +
5	0	2	+ + +	+ + +	+ + +	+ + +	+ +		+ +		+ +	+ +	+ +
6	0	2	+ +	0	+	+	0		0		0	0	0
7	0	2	+ +	0	+	+	0		0		0	0	+
8	0	2	+ +	0	+	+	0		0		0	0	+
9	20	1	+ +	0	+ +	+ + +	0		0		0	0	0
10	20	1	+ +	0	+ + +	+ + +	0		0		0	0	0
11	20	1	+ + +	+	+	+ + +	0		0		0	0	+
12	20	1	+ +	+	0	+	0		0		0	0	0
13	20	1	+ +	+	0	+	0		0		0	0	0
14	20	1	+ + +	+ +	+ + +	+ +	+		+		+	+	+ +