INFECTION AND IMMUNITY, Mar. 1979, p. 660-664
0019-9567/79/03-0660/05$02.00/0

Vol. 23, No. 3

Polymyxin B Sulfate Modification of Bacterial Endotoxin: Effects on the Development of Endotoxin Shock in Dogs

ARTHUR H. L. FROM,[1,2]† JACK S. C. FONG,‡ AND ROBERT A. GOOD§

Departments of Medicine (Cardiovascular Section),[1] Microbiology, Pathology and Laboratory Medicine, and Pediatrics, University of Minnesota, School of Medicine, Minneapolis, Minnesota 55455 and United States Veterans Administration Medical Center, Minneapolis, Minnesota 55417[2]

Received for publication 18 December 1978

The effects of endotoxin (lipopolysaccharide [LPS]) on the pathogenesis of canine endotoxin shock were compared with those of LPS which had interacted with polymyxin B sulfate prior to administration. Both LPS and polymyxin B-modified LPS caused comparable early decreases in aortic blood pressure, leukocyte and platelet numbers, and serum complement levels. However, in dogs receiving polymyxin B-modified LPS the late hypotensive phase was significantly ameliorated and lethality was significantly decreased. These data indicate that polymyxin B-modified LPS, though significantly less lethal than unmodified LPS, was capable of major interactions with several components of the humoral defense system, and support the concept that such interactions are not determinative in the pathogenesis of canine endotoxin shock.

Polymyxin B sulfate (PMB), a cyclic polypeptide antibiotic, interacts with gram-negative bacterial endotoxin (lipopolysaccharide [LPS]) with a resultant loss of many of the biological properties of LPS (6, 7, 16-18). PMB-modified LPS does not generate a generalized Shwartzman reaction in prepared rabbits (6), is considerably less lethal than unmodified LPS in chicken embryos (18) and mice (17), and decreases mortality in rabbits made septic with PMB-resistant gram-negative organisms (J. J. Corrigan, Jr. and J. F. Kiernat, Pediatr. Res. 11: 498, 1977). Because of these interesting observations, a canine endotoxin shock model was utilized to compare and contrast the humoral and hemodynamic effects of PMB-modified LPS and unmodified LPS with the goal of gaining more insight into the mechanisms of importance in the pathogenesis of endotoxin shock.

MATERIALS AND METHODS

Mongrel dogs of either sex weighing 10 to 12 kg were anesthetized with pentobarbitol (30 mg/kg) administered intravenously and supplemented as needed. Aortic pressure was monitored continuously during each experiment with a strain-gauge transducer via a catheter inserted in the femoral artery and advanced to the thoracic aorta; a femoral vein catheter was inserted for blood sampling and drug infusion. Pressure was recorded with an oscillographic recording system, and mean pressure was obtained electronically.

Blood samples were obtained for the measurement of leukocyte (WBC), platelet (P), and serum complement (C) levels, which were carried out as previously described (10, 11), and blood volumes removed for sampling were replaced with sterile saline. In each experiment, control measurements of each variable were obtained 30 to 60 min prior to any experimental manipulation, and then sequentially over a 4-h observation period after LPS administration. After the observation period, catheters were removed, the catheter insertion sites were dressed, and the animals were further observed, during which period they had free access to food and water. Survival in these experiments was defined as an animal living for 72 h.

Four groups of dogs were studied: (i) seven dogs that received 0.9 mg of *Escherichia coli* LPS per kg (a previously determined 80% lethal dose) (10, 11) injected as an intravenous bolus; (ii) seven dogs that received a mixture of 0.9 mg of LPS and 5 mg of PMB (Burroughs-Wellcome Co., Tuckahoe, N.Y.) per kg, dissolved in sterile 5% dextrose solution, which was preincubated for 15 min and then injected as an intravenous bolus; (iii) seven dogs in which 5 mg of PMB per kg was infused over 30 min beginning 60 min before the intravenous bolus injection of 0.9 mg of LPS per kg; and (iv) six dogs that received a 30-min infusion of 5 mg of PMB per kg, beginning 1 h after the intravenous bolus injection of 0.9 mg of LPS per kg.

The amount of PMB used to modify LPS effects was chosen on the basis of data indicating that a PMB-LPS ratio of greater than 5:1 neutralized many of the biological effects of LPS in rabbits (6).

The major technical problems in this study related to two toxic properties of PMB: (i) neuromuscular

† Send reprint requests to: U.S. Veterans Administration Medical Center, Minneapolis, MN 55417.

‡ Present address: The Montreal Children's Hospital, Montreal, Quebec, H3H1P3, Canada.

§ Present address: Sloan-Kettering Institute for Cancer Research, New York, NY 10021.

blockade induced by the drug, which may cause transient respiratory arrest (1), and (ii) the ability of PMB to cause histamine release from the mast cell with resulting hypotension (5). The former problem was controlled by intubating all groups of dogs with an endotrachial tube and respiring (Harvard Instrument respiratory pump) those that suffered transient respiratory difficulties after PMB injection until they were capable of unassisted ventilation (usually 40 to 60 min after PMB-LPS mixture injection and for longer periods or throughout experiment when PMB was injected before or after endotoxin). The respiratory arrest was manifested by diminution of the strength of the respiratory effort, not the rate, and occurred within 2 to 5 min following PMB injection. Since respiratory arrest can be a manifestation of endotoxin shock in dogs, and indeed occurred in several control experiments several hours after LPS injection, these control dogs were also ventilated so as not to give the PMB-treated dogs a possible survival advantage. No dog was mechanically ventilated for more than 4 h after LPS injection.

With respect to the histamine-releasing properties of PMB, no effort was made at compensation through the use of antihistaminics since it was felt that such drug intervention might in some way alter the course of the experiments.

All results are expressed a mean ± standard error of the mean. Statistical analyses were carried out utilizing Student's t test (paired or unpaired as appropriate) and the corrected chi-square test; the significance level accepted was $P < 0.05$.

RESULTS

The hemodynamic findings (Fig. 1A) after LPS injection are typical with an immediate marked drop in mean aortic blood pressure, followed by a partial recovery over 30 min, a late drop in pressure, and then later recovery. When PMB-modified LPS was compared with LPS (Fig. 1A), the secondary drop in mean aortic pressure was absent, though the immediate fall was preserved. Infusion of PMB before or after LPS challenge (Fig. 1B and C) did not modify the basic hemodynamic response to LPS, though in the preinfusion experiments, a persistent primary hypotensive effect of PMB was present and continued after cessation of the infusion. This response was not apparent in the PMB postinfusion experiments, but could be masked by the significant post-LPS hypotension present at that time. The differences in mean aortic pressure between the LPS and PMB-LPS groups (Fig. 1A) were statistically significant between 90 and 180 min but not at the termination of observation, presumably because the two most hypotensive animals in the control group had already died. In Fig. 1B it can be seen that PMB infusion alone produced a significant persistent fall in aortic blood pressure, but by 150 min post-LPS there were no time periods when the treatment group differed from the

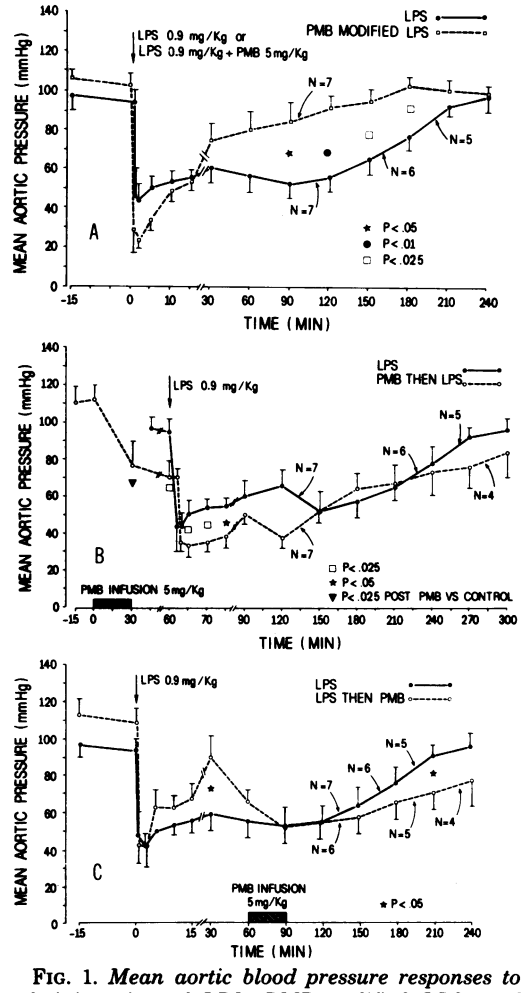

FIG. 1. *Mean aortic blood pressure responses to administration of LPS, PMB-modified LPS, and PMB.*

control group.

The marked fall in WBC numbers caused by LPS alone also occurred with PMB-modified LPS (Fig. 2A), and it can be seen (Fig. 2B) that PMB infusion alone caused a significant persistent fall in WBC numbers, which fell further with LPS. PMB infusion post-LPS (Fig. 2C) did not modify already low WBC levels. The marked fall in P numbers post-LPS also occurred with PMB-modified LPS (Fig. 3A); pre- or post-LPS infusion of PMB did not itself substantially alter P numbers or the response of P to LPS (Fig. 3B and C).

C levels (Table 1), which were not available in every animal, fell significantly after LPS and PMB-modified LPS, but when LPS was given after PMB the fall in C was not significant, though in this group, C levels in five of six animals dropped. Infusion of PMB alone did not

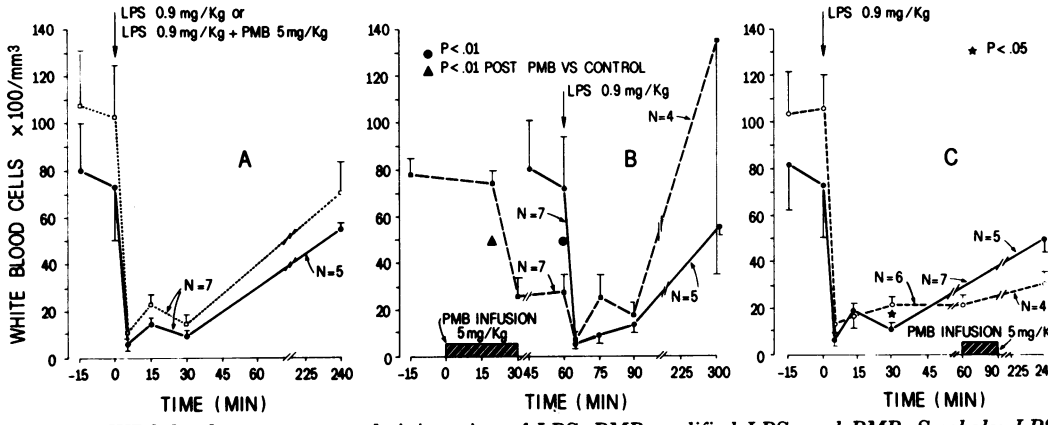

FIG. 2. *WBC level responses to administration of LPS, PMB-modified LPS, and PMB. Symbols: LPS (●——●); LPS then PMB (○---○); PMB then LPS (■——■); PMB-modified LPS (□-----□).*

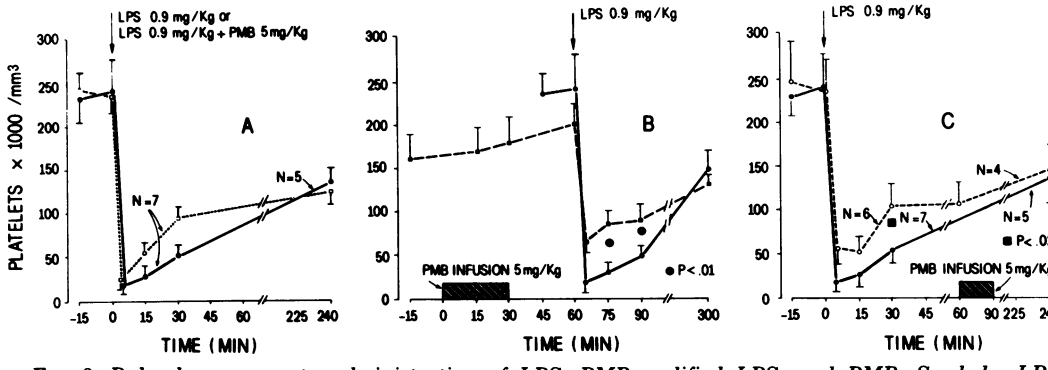

FIG. 3. *P level responses to administration of LPS, PMB-modified LPS, and PMB. Symbols: LPS (●——●); LPS then PMB (○---○); PMB then LPS (■——■); PMB-modified LPS (□-----□). Bars indicate standard error.*

TABLE 1. *Effect of LPS, PMB-modified LPS, and LPS post-PMB infusion on serum complement levels*

Treatment	No. of dogs	Serum complement level[a] at time:		
		0 min (control)	5 min post-LPS	30 min post-LPS
LPS	4	29 ± 6.0	15 ± 4.2[b]	15 ± 3.7[b]
PMB-modified LPS	5	45 ± 5.0	35 ± 2.3[c]	30 ± 4.0[c]
LPS post-PMB	6	46 ± 7.2	39 ± 17.1[d]	41 ± 21.9[d]

[a] Expressed as mean 50% hemolytic complement units ± standard error.

[b] Difference between 5-min and 30-min samples and 0-min sample was significant ($P < 0.005$).

[c] Difference between 5-min and 30-min samples and 0-min samples was significant ($P < 0.025$ and 0.005, respectively).

[d] Differences between 5-min and 30-min samples and 0-min samples were not significant.

affect C levels (data not shown).

In the LPS group, the mortality was six of seven dogs (all dying within 24 h post-LPS, and two within 4 h post-LPS). The mortality of the PMB-modified LPS group was one of seven, with the lone animal dying at 36 h post-LPS. The difference in mortality between these two groups was significant ($P < 0.05$). The mortality of the PMB-infusion-followed-by-LPS group was four of seven, and that of the LPS-followed-by-PMB-infusion group was four of six, neither of which was significantly different from the LPS control group.

DISCUSSION

The data presented demonstrate that (i) PMB-modified LPS is significantly less lethal than the same quantity of unmodified LPS in a canine endotoxin shock model; (ii) pre- or post-treatment of dogs with PMB does not significantly decrease the mortality of unmodified LPS; and (iii) PMB-modified LPS maintains its vigorous interaction with several components of the humoral defense system (WBC, P, and C) and induces an initial hemodynamic response comparable to that of unmodified LPS.

Consistent with these observations, Palmer

and Rifkind (16) found that PMB-modified LPS did not cause the late (90 min or later) hemodynamic changes seen with the same sublethal dose of unmodified LPS. Furthermore, Craig et al. reported that pretreatment of dogs with PMB did not prevent LPS lethality (8). Similarly, Corrigan and Bell demonstrated that PMB-modified LPS did not elicit the Shwartzman reaction in prepared rabbits, but that PMB pre- or posttreatment of rabbits was relatively ineffective in modifying the generalized Shwartzman reaction unless administered within 15 min of the LPS challenge dose (6).

Possible explanations of effects of PMB on LPS toxicity include (i) direct interaction between PMB and LPS such that LPS can no longer interact effectively with humoral defense systems or target tissue "receptors," and (ii) blockade of LPS receptor sites by PMB such that they cannot interact with LPS. The lack of effect of pre- or posttreatment of dogs with PMB noted in this study and by others (6, 8) supports the concept that PMB acts primarily to modify LPS rather than the host response to LPS. Biochemical studies are also consistent with such an interaction in that it has been shown that PMB interacts with bacterial cell membrane components with a resultant breakdown of the cellular permeability barrier and consequent cell death (2, 15). Whether free PMB blockade of LPS cellular attachment sites makes an additional contribution to the protection induced by PMB, as has been recently postulated (3), is unknown.

In the present study, of the major variables measured in addition to lethality, only the late hypotensive response seen following PMB-modified LPS seemed blunted, and falls in WBC and P numbers and C levels were unaltered. PMB infusion prior to LPS injection did decrease the WBC number and mean aortic blood pressure significantly, but the response to subsequent LPS challenge was basically unaltered with respect to the humoral (other than the post-LPS C response) or hemodynamic changes (Fig. 1 to 3 and Table 1), and mortality was not diminished. Because PMB-modified LPS was capable of effective interaction with several components of the humoral defense system and in spite of this lethality was decreased, the characteristic rapid massive activation of these components would not seem to be the determinative mechanism in the pathogenesis of canine endotoxin shock.

Consistent with this view are our earlier observations that neither pre-existing thrombocytopenia (9) nor prevention of disseminated fibrin clot formation by prior Arvin-induced defibrination (11) blocks the genesis of canine endotoxin shock. We have also found that, though prior decomplementation does modify the initial "anaphylactoid" response of dogs to LPS, mortality is not altered (10). Further, Spink and associates (20) have shown in dogs immunized against *E. coli* LPS that the initial or anaphylactoid (humoral) response was actually enhanced following LPS rechallenge, though the lethality of the dose (an 80% lethal dose) was markedly reduced.

With respect to the possibility that primary LPS-tissue interactions can occur, Rubinstein and associates (19) have found that intravenously injected LPS deposits widely in the vascular system of intact dogs, frequently being found in the endothelium. In addition, other workers have shown that LPS induces significant permeability changes in the alveolar capillary bed of a perfused canine lung preparation even in the absence of C or other mediators in the perfusing medium (4). These observations suggest a role in the pathogenesis of endotoxin shock for direct LPS-tissue interactions. The possibility that such interactions could be blocked by prior treatment of LPS with PMB is supported by the finding of Neter and associates (14) that LPS pretreated with PMB attached poorly to the erythrocyte surface, in marked contrast to untreated LPS. Prevention of LPS lethality in animals passively or actively immunized against LPS (20, 21) also may relate to the inability of LPS bound to antibody to attach to tissue sites.

The persistence of marked alterations in WBC, P, and C levels after infusion of PMB-modified LPS bears further comment in view of contrary observations in other species (3, 7). Since PMB is felt to interact primarily with the toxic lipid portion of LPS (lipid A) (2), the ability of PMB-modified LPS to interact with WBC, P, and C may depend upon some structural or biochemical property of LPS not modified by PMB, or to an intrinsic property of the PMB-LPS complex analogous to aggregated gamma globulin activation of C (13) or the more recently described reactivity of heparin-protamine complexes (12). An alternative explanation is that the PMB-modified LPS contained free LPS (or dissociated to release LPS) in adequate quantities to trigger the humoral response, although the PMB-LPS dissociation rate appears to be low under physiological conditions (2). The latter explanation has been advanced to explain residual PMB-modified LPS interaction with human and rabbit WBC, species in which the modified LPS is markedly less reactive with respect to the WBC (7). However, even if this is correct, the data demonstrate a clear dissociation between humoral and lethal effects of LPS.

ACKNOWLEDGMENTS

We wish to express our gratitude to Wesley W. Spink for the gift of the endotoxin used in these studies, to Pat Gerardy for her excellent technical assistance, and to Irene Linc for her skillful aid in preparation of this manuscript.

This work was supported by grants from the Minnesota Medical Foundation, the American Heart Association, Minnesota Affiliate, National Institutes of Health (Public Health Service grants no. AI-11843-02 and NS-11457), and Veterans Administration research funds.

LITERATURE CITED

1. **Adamson, R. H., F. N. Marshall, and J. P. Long.** 1960. Neuromuscular blocking properties of various polypeptide antibiotics. Proc. Soc. Exp. Biol. Med. **105**:494–497.
2. **Bader, J., and M. Teuber.** 1973. Action of polymyxin B on bacterial membranes. 1. Binding to the O antigenic lipopolysaccharide of *Salmonella typhimurium*. Z. Naturforsch. **28c**:422–430.
3. **Bannatyne, R. M., W. M. Harnett, K.-Y. Lee, and W. D. Biggar.** 1977. Inhibition of the biologic effects of endotoxin on neutrophils by polymyxin B sulfate. J. Infect. Dis. **136**:469–474.
4. **Bessa, S. M., A. P. Dalmasso, and R. L. Goodale, Jr.** 1974. Studies on the mechanism of endotoxin induced increase of alveolocapillary permeability. Proc. Soc. Exp. Biol. Med. **147**:701–705.
5. **Bushby, S. R. M., and A. F. Green.** 1955. The release of histamine by polymyxin B and polymyxin E. Br. J. Pharmacol. **10**:215–219.
6. **Corrigan, J. J., Jr., and B. M. Bell.** 1971. Endotoxin induced intravascular coagulation: prevention with polymyxin B sulfate. J. Lab. Clin. Med. **77**:802–810.
7. **Corrigan, J. J., Jr., O. F. Sieber, Jr., H. Ratajczak, and B. B. Bennett.** 1974. Modification of human neutrophil response to endotoxin with polymyxin B sulfate. J. Infect. Dis. **130**:384–387.
8. **Craig, W. A., J. H. Turner, and C. M. Kunin.** 1974. Prevention of generalized Shwartzman reaction and endotoxin lethality by polymyxin B localized in tissues. Infect. Immun. **10**:287–292.
9. **From, A. H. L., J. S. C. Fong, T. Chiu, and R. A. Good.** 1976. The role of platelets in the pathogenesis of canine endotoxin shock. Infect. Immun. **13**:1591–1594.
10. **From, A. H. L., H. Gewurz, R. P. Gruninger, R. J. Pickering, and W. W. Spink.** 1970. Complement in endotoxin shock: effect of complement depletion on early hypotensive phase. Infect. Immun. **2**:38–41.
11. **From, A. H. L., W. W. Spink, D. G. Knight, and H. Gewurz.** 1975. Significance of intravascular coagulation in canine endotoxin shock. Infect. Immun. **11**:1010–1013.
12. **Gewurz, H., and N. Ertel.** 1973. Non-immune activation of complement: two new phenomena, p. 340–349. *In* W. Brann and J. Ungar (ed.), Non-specific factors influencing host resistance. Karger, Basel.
13. **Ishizaka, T., and K. Ishizaka.** 1959. Studies of aggregated gamma globulin. 1. Sedimentation, electrophoretic and anticomplementary properties. J. Immunol. **84**:112–120.
14. **Neter, E., E. A. Gorzynski, O. Westphal, and O. Lüderitz.** 1958. The effects of antibiotics on enterobacterial lipopolysaccharides (endotoxins), hemagglutination and hemolysis. J. Immunol. **80**:66–72.
15. **Newton, B. A.** 1956. The properties and mode of action of the polymyxins. Bacteriol. Rev. **20**:14–27.
16. **Palmer, J. D., and D. Rifkind.** 1974. Neutralization of the hemodynamic effects of endotoxin by polymyxin B. Surg. Gynecol. Obstet. **138**:755–759.
17. **Rifkind, D.** 1967. Prevention by polymyxin B of endotoxin lethality in mice. J. Bacteriol. **93**:1463–1464.
18. **Rifkind, D., and J. D. Palmer.** 1966. Neutralization of endotoxin toxicity in chick embryos by antibiotics. J. Bacteriol. **92**:815–819.
19. **Rubinstein, H. S., J. Fine, and A. H. Coons.** 1962. Localization of endotoxin in the walls of the peripheral vascular system during endotoxinemia. Proc. Soc. Exp. Biol. Med. **111**:458–467.
20. **Spink, W. W., R. B. Davis, R. Potter, and S. Chartrand.** 1964. The initial stage of canine endotoxin shock as an expression of anaphylactic shock: studies on complement titers and plasma histamine concentrations. J. Clin. Invest. **43**:696–704.
21. **Ziegler, E. J., H. Douglas, and A. I. Braude.** 1973. Human antiserum for prevention of the local Shwartzman reaction and death from bacterial lipopolysaccharides. J. Clin. Invest. **52**:3236–3238.