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Activity of Lipopolysaccharide-Binding Protein–Bactericidal/ Permeability-Increasing Protein Fusion Peptide in an Experimental Model of *Pseudomonas* Sepsis

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A chimeric protein consisting of the N-terminal domain of lipopolysaccharide-binding protein and the C-terminal domain of bactericidal/permeability-increasing protein demonstrated a dose-dependent survival benefit ($P = 0.001$) and reduced endotoxin levels ($P < 0.01$) in neutropenic rats with *Pseudomonas aeruginosa* sepsis. This lipopolysaccharide-binding protein–bactericidal/permeability-increasing peptide has favorable pharmacokinetics and antiendotoxin properties which may be of value for human sepsis.

Current therapeutic options for gram-negative bacterial sepsis are limited to antimicrobial agents, hemodynamic support, and management of sepsis-induced organ dysfunction (4). Efforts to interfere directly in the pathophysiologic mechanisms which underlie the septic process have yielded inconsistent and largely disappointing results. Antiendotoxin monoclonal antibodies (11, 18), anticytokine therapies (1, 6, 8), and other anti-inflammatory strategies (5, 24) have not proven to be of sufficient benefit to warrant approval as standard adjunctive therapies for human sepsis.

Despite these setbacks, it is anticipated that refinements in clinical trial design and innovations in the synthesis of more potent therapeutic agents will lead to significant advances in the treatment of sepsis in the future. A naturally occurring endotoxin-binding and neutralizing protein which may prove to be particularly effective in endotoxemic states is bactericidal/permeability-increasing protein (BPI) (17, 25). This cationic human neutrophil-derived, 456-amino-acid protein is known to possess potent endotoxin neutralizing properties and intrinsic antimicrobial actions (16, 20, 26). It has recently been shown that BPI is protective in a variety of endotoxin challenge experiments both in vitro (3, 9, 19) and in vivo (2, 7, 16).

A potential limitation to the therapeutic utility of BPI in clinical endotoxic shock is its rapid clearance from the central circulation, with a plasma half-life of only 2 to 4 min in experimental animals (7). A recombinant fusion construct of human BPI with a closely related endotoxin-binding protein, known as lipopolysaccharide (LPS)-binding protein (LBP) (23, 27), has been generated; this combines the endotoxin-neutralizing properties of BPI with the favorable clearance properties of LBP. BPI and LBP are genetically (10) and structurally (26) related proteins, yet they have opposing physiologic actions in the presence of LPS (12, 22). BPI inhibits interactions between LPS and CD14-bearing effector cells such as neutrophils and monocytes. LBP, in contrast, facilitates the delivery of LPS to CD14 antigens on cell membranes and potentiates LPS activity.

A chimeric protein consisting of the first 199 amino acids of the amino terminus of LBP and the C-terminal 257 amino acids of BPI was found to possess desirable attributes of both LBP and BPI in endotoxin challenge models (15). This fusion peptide was tested in a bacteremic infection model of *Pseudomonas aeruginosa* sepsis in neutropenic rats.

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The LBP-BPI fusion protein was provided as a gift from Incyte Pharmaceuticals (Palo Alto, Calif.) and was stored at −70°C. The protein was prepared in sterile saline without vortexing prior to its intravenous administration at doses ranging from 1 to 5 mg/kg of body weight per animal. The fusion peptide was given intravenously via the rat tail vein at the onset of fever in neutropenic rats. An irrelevant murine immunoglobulin M monoclonal antibody (B55) was given as a placebo at 1 mg/kg intravenously to the control group. The placebo and LBP-BPI fusion peptide were given daily for 3 days. The details of the neutropenic rat model have been described previously (21). The Sprague-Dawley, specific-pathogen-free, female albino rats (Charles River Laboratory, Wilmington, Mass.) were rendered neutropenic with cyclophosphamide (Sigma, St. Louis, Mo.) at time zero (150 mg/kg given intraperitoneally and 72 h later (50 mg/kg given intraperitoneally). Animals were challenged with *P. aeruginosa* 12.4.4 (Fisher-Devlin-Grabasik immunotype VI) by orogastric feeding with approximately 10^7 CFU in 1 ml of phosphate-buffered saline (PBS) at 0, 2, and 4 days after cyclophosphamide treatment. Blood samples were taken from each animal at the baseline (day −2), at the onset of fever but before treatment (day 5), and 24 h after the first treatment (day 6). The animal model and study protocol were reviewed and approved by the Institutional Animal Care Committee at Brown University prior to the initiation of these studies.

Quantitative bacterial counts, *Limulus* amebocyte lysate assays (Associates of Cape Cod, Woods Hole, Mass.), and the L929 cytotoxicity assays for tumor necrosis factor alpha (TNF-α) levels (13) were performed for each animal. Necropsy examination was performed on all animals that succumbed during the course of the 12-day experiment. Detailed histologic examination of liver, splenic, lung, renal, and adrenal tissue was conducted along with quantitative measurement of bacterial counts from liver, lung, and splenic tissues.

Differences in survival rates at the end of the experiments

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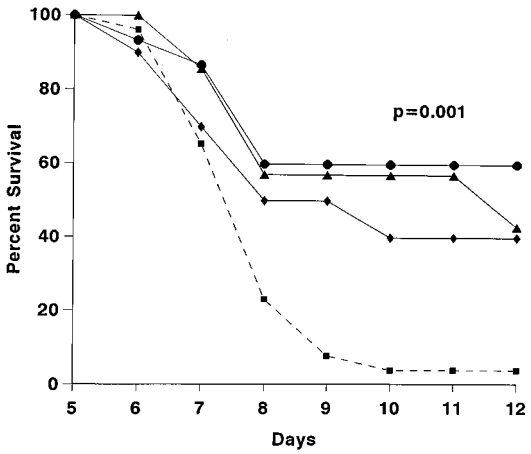

FIG. 1. Survival rates of neutropenic rats with increasing doses of LBP-BPI fusion peptide treatment compared with survival rates of the control group. The LBP-BPI fusion peptide was administered at 1 mg/kg (◆; $n = 10$), 2 mg/kg (▲; $n = 7$), and 5 mg/kg (●; $n = 15$). ■, control group ($n = 26$).

were calculated by using chi-square analysis-of-contingency tables for multiple groups. Continuous variables for multiple groups were compared by the Kruskal-Wallis test with Dunn's multiple comparisons test. In order to obtain normally distributed data for endotoxin levels, the data were analyzed by using logarithmic transformations of the measured values. Determinations of the logarithmic mean and 95% confidence intervals were then expressed as geometric means and confidence intervals by antilogarithmic conversions. An analysis of variance followed by the Tukey-Kramer test for multiple comparisons was then performed.

A summary of the survival rates from the dose escalation experiments is depicted in Fig. 1. The placebo-treated animals ($n = 26$) developed a multisystem infection with the challenge strain of *P. aeruginosa* which was lethal to 96% of these neutropenic animals within 96 h of its onset. Histologic examination revealed acute pulmonary congestion, diffuse interstitial edema, and acute tubular necrosis without evidence of discrete tissue abscess formation. Increasing doses of the LBP-BPI fusion peptide led to progressive improvements in survival, with up to 60% of animals assigned to the highest treatment dose (5 mg/kg for 3 days) surviving the entire neutropenic period.

LBP-BPI fusion peptide treatment resulted in significant dose-dependent reductions in measurable endotoxin levels (Pearson correlation coefficient $r = -0.43$; $P = 0.0012$) when compared with those in the control group after 24 h of treatment (Fig. 2). The frequency of bacteremia (91% overall; range, 86.5 to 100%) and the quantitative level of bacteremia (101 ± 42 CFU/ml) did not differ between each of the LBP-BPI-treated groups and the control group before the initiation of therapy. The LBP-BPI fusion peptide did not possess any detectable antimicrobial activity against the challenge organism by broth dilution MIC testing up to 50 mg of LBP-BPI per ml in Mueller-Hinton broth alone or in broth supplemented with 10% rat serum.

No reduction in the quantitative level of bacteremia was observed between each treatment group and the control group after 24 h of therapy. The average quantitative blood culture counts in the treatment groups ranged from 81 to 420 CFU/ml and did not differ from that in the control group (249 ± 198 CFU/ml). Quantitative colony counts of the challenge strain of *P. aeruginosa* 12.4.4 from lung, liver, and splenic tissue did not significantly differ between LBP-BPI-treated animals and the control group (data not shown). TNF-α levels were low (<20 pg/ml) in all animals studied and did not significantly differ between the treatment groups and the placebo group.

These results indicate that this chimeric fusion peptide provides a survival benefit in an actual infection model of gram-negative bacteremia in immunocompromised animals. This model mimics the sequence of events which often occur clinically in septic patients with chemotherapy-induced neutropenia. The activity of the LBP-BPI fusion construct in this model would suggest that this antiendotoxin strategy might be of potential utility in clinical medicine. However, the limitations of animal models should be carefully considered before estimates of the potential clinical efficacy of the construct in human sepsis are attempted.

The fusion peptide has an expanded duration of activity within the central circulation with a clearance rate which is 75-fold slower (0.175 ml/min) than that of BPI itself (13 ml/min) (15). This should allow for extended dosing intervals with prolonged antiendotoxin effects. This pharmacokinetic property is advantageous clinically in that it provides much needed dosing flexibility in the treatment of septic patients.

The LBP-BPI fusion peptide was effective in reducing endotoxin levels and mortality in experimental animals, despite any demonstrable antibacterial activity of the peptide. Little et al. (14) have recently shown that the bactericidal activity of BPI appears to be limited to a short sequence of amino acids located between 85 and 99 amino acids from the N terminus of BPI. This bactericidal peptide region is not present in the LBP-BPI fusion peptide described here.

The study of genetically engineered constructs of LBP-BPI hybrid molecules should lead to a greater understanding of the structure-function relationships between these two important human endotoxin-binding proteins. The efficacy of this fusion peptide supports the hypothesis that the potent endotoxin-neutralizing properties of BPI rather than its intrinsic bactericidal properties are primarily responsible for the therapeutic activity of the protein (15). These results also support the notion that endotoxin neutralization alone is sufficient to be of therapeutic value in systemic infections with gram-negative bacteria. This potential treatment strategy should continue to be evaluated clinically as more effective antiendotoxin agents are developed.

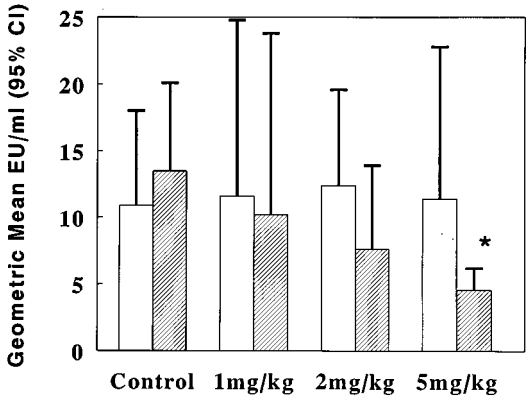

FIG. 2. Geometric mean endotoxin levels by treatment group before therapy (day 5; open bars) and 24 h after the first LBP-BPI fusion peptide treatment (day 6; slashed bars). No differences were found between groups before therapy; *, endotoxin levels were significantly lower after therapy in the 5-mg/kg group compared with those in the control group ($P < 0.01$; corrected for multiple comparisons). EU, endotoxin units; CI, confidence intervals.

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