

Phase 2 trial of eritoran tetrasodium (E5564), a Toll-like receptor 4 antagonist, in patients with severe sepsis*

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Objectives: Endotoxin is a potent stimulus of proinflammatory response and systemic coagulation in patients with severe sepsis. Endotoxin is a component of Gram-negative bacteria that triggers an innate immune response through Toll-like receptor 4 signaling pathways in myeloid cells. We evaluated safety and tolerability of two dose regimens of eritoran tetrasodium (E5564), a synthetic Toll-like receptor 4 antagonist, and explored whether it decreases 28-day mortality rate in subjects with severe sepsis.

Design: Prospective, randomized, double-blind, placebo-controlled, multicenter, ascending-dose phase II trial.

Setting: Adult intensive care units in the United States and Canada.

Patients: Three hundred adults within 12 hrs of recognition of severe sepsis, with Acute Physiology and Chronic Health Evaluation (APACHE) II-predicted risk of mortality between 20% and 80%.

Interventions: Intravenous eritoran tetrasodium (total dose of either 45 mg or 105 mg) or placebo administered every 12 hrs for 6 days.

Measurements and Main Results: Prevalence of adverse events was similar among subjects treated with 45 mg or 105 mg of

eritoran tetrasodium or with placebo. For modified intent-to-treat subjects, 28-day all-cause mortality rates were 26.6% (eritoran tetrasodium 105 mg), 32.0% (eritoran tetrasodium 45 mg), and 33.3% in the placebo group. Mortality rate in the eritoran tetrasodium 105-mg group was not significantly different from placebo ($p = .335$). In prespecified subgroups, subjects at highest risk of mortality by APACHE II score quartile had a trend toward lower mortality rate in the eritoran tetrasodium 105-mg group (33.3% vs. 56.3% placebo group, $p = .105$). A trend toward a higher mortality rate was observed in subjects in the lowest APACHE II score quartile for the eritoran 105-mg group (12.0% vs. 0.0% placebo group, $p = .083$).

Conclusions: Eritoran tetrasodium treatment appears well tolerated. The observed trend toward a lower mortality rate at the 105-mg dose, in subjects with severe sepsis and high predicted risk of mortality, should be further investigated. (Crit Care Med 2010; 38:72–83)

KEY WORDS: severe sepsis; eritoran tetrasodium; E5564; Toll-like receptor; sepsis; endotoxin antagonist

Endotoxin (lipopolysaccharide) is the major constituent of the outer membrane of both Gram-negative pathogenic bacteria and normal enteric Gram-negative flora. Endotoxin, when administered intravenously to healthy volunteers, stimulates proinflammatory and thrombotic responses observed in severe sepsis patients (1, 2). Patients with severe sepsis initiated by Gram-negative organ-

isms, Gram-positive organisms, and fungi have high plasma levels of endotoxin (3). Release of endogenous endotoxin from the gut and liver due to splanchnic hypoperfusion is thought to explain this phenomenon (3). Sepsis patients with high levels of plasma endotoxin activity have an increased mortality rate (1). Although the immune response initiated by endotoxin may be protective and act as an early warning sign of microbial invasion,

the adverse effects of widespread inflammation, coagulopathy, and vascular compromise seen in severe sepsis are often detrimental. Endotoxin is transferred from the bacterial cell wall by a human lipopolysaccharide binding protein to membrane-bound CD-14 found on the cell surface of myeloid cells of the innate immune system. A signaling complex forms, consisting of endotoxin, an adaptor protein (MD-2), and a transmembrane protein known as Toll-like receptor 4 (TLR4) (4–7). The lipid A portion of endotoxin binds to a complex of TLR4 and MD-2 via hydrophobic and electrostatic forces (8, 9). Subsequent dimerization of these complexes of endotoxin bound to TLR4-MD-2 leads to intracellular signaling, production of nuclear factor- κ B, and ultimately proinflammatory cytokines.

In the past, therapies designed to interfere with endotoxin did not improve outcome (10–21). However, new therapeutic targets are suggested by recent discoveries that reveal how endotoxin

***See also p. 306.**

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triggers cell signaling and inflammation by interacting with TLR4.

Eritoran tetrasodium (E5564) is a synthetic lipopolysaccharide designed to interfere with endotoxin signaling via the TLR4 receptor. The structure of the molecule is based on the lipid A portion of a naturally occurring, weakly agonistic endotoxin found in *Rhodobacter sphaeroides* (22–25). Eritoran tetrasodium is a potent *in vitro* antagonist of endotoxin (24, 25) that directly binds to the hydrophobic pocket of MD-2, competitively inhibits the lipid A component of endotoxin from binding to the same site, and thereby prevents dimerization of TLR4 and intracellular signaling (4, 9).

Eritoran tetrasodium is significantly protective in animal models of sepsis (24). In a placebo-controlled human endotoxin challenge model in healthy volunteers, eritoran tetrasodium blocked the signs and symptoms of endotoxemia in a dose-dependent manner. Elevations in temperature, heart rate, white blood cell count, and serum levels of inflammatory markers (C-reactive protein, tumor necrosis factor- α , and interleukin [IL]-6) were reduced or prevented in eritoran tetrasodium-treated volunteers compared with placebo (26).

This randomized, double-blind, placebo-controlled phase II trial was designed to assess the safety and efficacy of eritoran tetrasodium in early severe sepsis and to determine a potential therapeutic dose. Pharmacokinetic modeling, based on the data from phase I studies, was used to select the two dose levels, the dosing schedule (every 12 hrs), and the 6-day treatment duration used in this study. This report summarizes the safety and efficacy outcomes for eritoran tetrasodium-treated patients with severe sepsis.

MATERIALS AND METHODS

Subjects

Between January 2002 and April 2005, we enrolled eligible adult patients from intensive care units in 99 hospitals in the United States and Canada. Independent ethics committees or institutional review boards at each study center approved the study protocol. Each subject, or a legally authorized surrogate, gave written informed consent. Eligible subjects were nonpregnant women and men aged 18 to 85 yrs with severe sepsis thought to be caused by bacterial or fungal pathogens. Study medication was to be administered within 12 hrs of

the recognition of severe sepsis. Eligible subjects had an Acute Physiology and Chronic Health Evaluation (APACHE) II score that predicted a risk of mortality within the range of 20% to 80%. APACHE II scores were determined using physiologic data from a 24-hr period before randomization, and risk of mortality was calculated from the score by applying weights based on intensive care unit admitting diagnosis (27).

Severe sepsis was defined as the presence of at least three of four criteria for systemic inflammatory response syndrome due to a presumed or known site of infection (Appendix 1) (28) in association with at least one of the following sepsis-induced organ dysfunctions: refractory shock, renal dysfunction, hepatic dysfunction, or metabolic acidosis (Appendix 2). Key exclusion criteria are listed in Appendix 3.

The primary efficacy end point was the 28-day all-cause mortality rate in the modified intent-to-treat (MITT) group. The MITT group included all randomized subjects who received any amount of eritoran tetrasodium or placebo.

A clinical evaluation committee determined a clinically evaluable population, a subset of the MITT population, after subjects completed 28 days of study but before data unblinding, based on six characteristics defined *a priori*: 1) subjects who were study drug compliant (received two loading doses and at least four maintenance doses or until resolution of all signs and symptoms of sepsis if this occurred earlier), including subjects who died or discontinued study drug for a serious adverse event (SAE) regardless of duration of dosing; 2) subjects who met all inclusion criteria, including those for organ failure and objective evidence of infection; 3) subjects who were given appropriate antibiotics and other sepsis therapy; 4) subjects who had a predicted mortality rate of 20% to 80% by APACHE II score at screening; 5) subjects who did not have major surgery through day 10 (this was for assessment of organ failure only); and 6) subjects who had no serious protocol violation.

Study Design

Study Drug and Administration. Eritoran tetrasodium was synthesized by Eisai Research Institute of Boston (Andover, MA) as previously described (22, 25). Two dose regimens were compared with placebo. The dose regimens were 45 mg or 105 mg total dose administered over 6 days. Both regimens were initiated with two loading doses, followed by nine maintenance doses, all given at 12-hr intervals. Both doses were anticipated to achieve plasma concentrations that exceeded the 100% inhibitory concentration of eritoran tetrasodium in *ex vivo* assays of endotoxin challenge and were $\geq 50\%$ less than the max-

imum dose previously tested in humans (29). The infusion volumes for the eritoran tetrasodium and placebo groups were identical regardless of the dose. Investigators were encouraged to deliver the drug only through a central venous catheter of a brand compatible with the formulation of the drug. Catheters with chlorhexidine-coated lumens were contraindicated.

Randomization and Blinding. This was a double-blind, placebo-controlled phase II clinical trial. Allocation concealment was maintained using a central phone office qualification and randomization center. To use the available drugs at each site efficiently, block randomization was conducted at a site level within the APACHE II-predicted mortality strata (low, 20% to 50%; high, 51% to 80%). Each site had two sequences of drug assignment based on APACHE II-predicted mortality rate: one sequence for subjects with predicted mortality rate 20% to 50%, and another sequence for subjects with predicted mortality rate 51% to 80%. This was done to yield a balanced allocation of high and low APACHE II-predicted mortality rate within the three treatment groups. The study was conducted in three stages to establish safety of a total dose of 45 mg of eritoran tetrasodium before proceeding to the higher dose of 105 mg. In the first stage, 50 subjects were randomized 1:1 to placebo or eritoran tetrasodium 45 mg, after which an independent data monitoring committee conducted a planned, grouped, but blinded interim safety analysis. In the second stage, 75 subjects were randomized (1:1:1) to placebo, eritoran tetrasodium 45 mg, or eritoran tetrasodium 105 mg, and another safety analysis was conducted. In the third stage, 175 subjects were randomized (2:2:3) to placebo, eritoran tetrasodium 45 mg, or eritoran tetrasodium 105 mg to attain 100 subjects per group for the entire study. Interval analyses by the data monitoring committee included mortality rate in each group without unblinding the groups. Unblinding would only have been done if there were statistical differences in groups, but there were none. The data monitoring committee adopted no predesignated *p* values for analyzing the 28-day SAE rate and used clinical judgment during review of the first 125 trial subjects. Because of multiple blinded looks, a small statistical penalty would have been taken on the composite *p* value if significant.

Sepsis Management. With the exception of the study drug infusion, treating physicians dictated care, including all decisions regarding the volume of fluid resuscitation given, choice and dosage of vasopressors, choice and duration of antibiotics, mechanical ventilation settings, use of corticosteroids and drotrecogin alfa (activated) (DAA; recombinant human activated protein C, Xigris, Eli Lilly & Co., Indianapolis, IN).

Data Collection. After informed consent, but before randomization, a physical examination, APACHE II score, documentation of in-

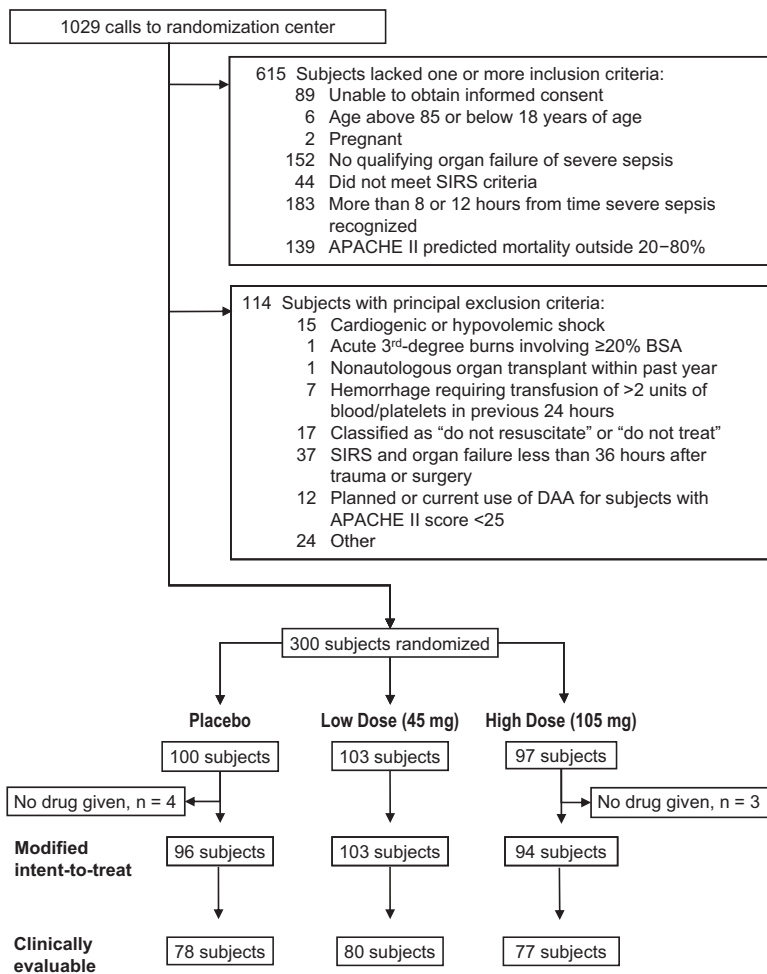


Figure 1. Flow diagram showing the total number of study participants with severe sepsis randomized to receive eritoran tetrasodium (45 mg or 105 mg) or placebo. Four patients randomized to placebo and three patients randomized to eritoran tetrasodium 105 mg did not receive any study medication. These seven patients were not included in the modified intent-to-treat population for analyses. *BSA*, body surface area; *SIRS*, systemic inflammatory response syndrome; *APACHE*, Acute Physiology and Chronic Health Evaluation; *DAA*, drotrecogin alfa (activated).

fection, and laboratory determinations (including human chorionic gonadotropin) were done. After randomization, but before study drug administration, the baseline data were collected. All patients were followed for the 28-day study period for organ failure, adverse events (AEs), and concomitant medications. Blood cultures were collected until eradication of pathogen was documented. Clinical laboratory tests were performed on days 1–10, 14, and 28, except for IL-6, which was collected on days 1–3. Daily arterial blood gas determinations were performed until subjects were removed from mechanical ventilation or day 28. All-cause mortality rate was determined on day 28.

Safety and Efficacy Measures

The safety and tolerability end points included the frequency of treatment-emergent AEs (TEAEs) and SAEs, clinically significant laboratory values, changes in vital signs, and physical examination findings. AEs and SAEs

were defined according to standard regulatory definitions. TEAEs were defined as events that were new in onset or aggravated in severity or frequency and abnormal results of diagnostic procedures between randomization and day 28 or the end of treatment. Abnormal laboratory values were considered AEs if they led to any type of intervention, as determined by investigators. A clinical evaluation committee reviewed all infectious AEs and classified these as either worsening of preexisting infection, a new infection (a distinct infectious episode after resolution of the sepsis-defining episode), or a superinfection (a distinct infectious episode beginning before the sepsis-defining infection had resolved).

Safety Laboratory Measures

Hematology and Chemistry Measurements. Local laboratories measured complete blood cell counts, coagulation tests, and se-

rum chemistry values at baseline and on days 1–10, 14, and 28.

Endotoxin and IL-6 Measurements. Samples for baseline measurement of endotoxin (whole blood, chromogenic Limulus amoebocyte lysate assay, detection limit 0.01 endotoxin unit (EU)/mL; Associates of Cape Cod, East Falmouth, MA) were collected in an endotoxin-free tube (Chromogenix, Franklin, OH), centrifuged to separate plasma, and frozen until analysis. Serum samples for endotoxin and for baseline IL-6 (baseline, 12 hrs, and 48 hrs after the start of study drug infusion; chemiluminescence assay, detection limit 5 pg/mL) were analyzed by a central referral laboratory (Specialty Laboratories, Valencia, CA).

E5564 Pharmacokinetics. Blood samples were obtained at three different time points to measure eritoran tetrasodium levels by reverse-phase liquid chromatography-tandem mass spectrometry assay for the purposes of performing population pharmacokinetic analysis (29). A sparse sampling design (three samples per patient) was obtained according to a randomization scheme throughout the 6 days of the dosing period and during the elimination phase up to 238 hrs after the last dose.

Statistical Analysis

Data were analyzed by a prospectively defined plan. Separate analysis compared mortality rates of subjects treated with placebo with mortality rates of a) all subjects treated with eritoran tetrasodium; b) subjects treated with low-dose (45 mg) eritoran tetrasodium; and c) subjects treated with high-dose (105 mg) eritoran tetrasodium. Statistical inference for the primary end point was based on the Cochran-Mantel-Haenszel (CMH) chi-square test. One subject who completed a treatment course of eritoran tetrasodium 105 mg refused study participation after day 8 but was known to be alive at day 28 and was included in the MITT analysis. Sample size selected for this study was anticipated to have a statistical power of 0.55 to detect a 15% difference in mortality rates. An explicit power calculation was not performed.

Six variables were identified *a priori* to define subgroups for further analysis of 28-day mortality rate: four APACHE II score quartiles and the presence of shock or absence of shock at baseline. The MITT population was divided into roughly equal quartiles based on APACHE II scores: quartile 1, score <21; quartile 2, score 21–24; quartile 3, score 25–28; quartile 4, score >28. An APACHE II score of 24.5 was the mean score for the MITT population. The APACHE II score quartiles and predicted mortality rate were correlated, in that for all patients with an APACHE II score ≥ 25 , predicted mortality rate was in the range of 51% to 80%. Statistical testing of subgroups compared mortality rate in subjects treated with placebo

Table 1. Baseline characteristics of modified intent-to-treat patients

Characteristic	Placebo (n = 96)	Eritoran Tetrasodium 45 mg (n = 103)	Eritoran Tetrasodium 105 mg (n = 94)	Overall <i>p</i> Value ^c
Mean age, yrs (SD)	60.6 (16.7)	57.5 (16.3)	59.1 (15.8)	.4020
Female gender, %	41.7	54.4	47.9	.2041
Caucasian race, %	77.1	68.0	73.4	.4116
Mean weight, kg (SD)	86.0 (28.0)	89.1 (38.0)	86.0 (24.8)	.7221
Mean APACHE II score (SD)	24.9 (5.8)	24.7 (5.5)	24.0 (5.2)	.5736
Mean predicted mortality rate by APACHE II score, % (SD)	53.1 (18.6)	52.6 (18.0)	52.5 (17.6)	.9711
Met SIRS criteria at baseline, %				
Heart rate	95.8	96.1	94.7	.8810
Respiratory	90.6	92.2	94.7	.5508
Temperature	70.8	70.9	72.3	.9729
White blood count	88.5	79.6	70.2	.0072
SOFA cardiovascular component ≥ 2 , %	72.9	76.7	80.9	.4342
Organ failure qualifying subject for study, %				.9076
Refractory shock	45.8	52.4	52.1	
Respiratory failure	20.8	17.5	19.1	
Acute renal failure	21.9	17.5	20.2	
Acute metabolic acidosis	11.5	12.6	8.5	
Acute liver dysfunction	0	0	0	
Number of organ failures, n (%) ^a				.0502
0	0 (0.0)	1 (1.0)	1 (1.1)	
1	23 (24.0)	14 (13.6)	15 (16.0)	
2	27 (28.1)	38 (36.9)	35 (37.2)	
3	25 (26.0)	36 (35.0)	25 (26.6)	
4	19 (19.8)	8 (7.8)	10 (10.6)	
5	2 (2.1)	4 (3.9)	7 (7.4)	
6	0 (0.0)	2 (1.9)	1 (1.1)	
Day 0 detectable but not elevated endotoxin level (%) ^b	22	28	21	
Day 0 detectable and elevated endotoxin level (%) ^b	69	66	77	.2160
Mean day 0 serum IL-6, pg/mL (SD)	27,192 (99,329)	16,667 (75,074)	37,789 (124,806)	.3503
Concomitant therapies				
Drotrecogin alfa (activated), n (%)	16 (16.7)	26 (25.2)	16 (17.0)	.2428
Systemic corticosteroids, n (%)	50 (52.1)	59 (57.3)	56 (59.6)	.5459

APACHE, Acute Physiology and Chronic Health Evaluation; SIRS, systemic inflammatory response syndrome; SOFA, Sequential Organ Failure Assessment; IL, interleukin.

^aNumber of patients with each sum (1–6) of the number of organ failures, where organ failure is defined as a SOFA score ≥ 2 for an organ system (cardiovascular, respiratory, liver, renal, coagulation, central nervous system); ^bEndotoxin was detectable in the range of 0.01–0.2 endotoxin units (EU)/mL. Endotoxin levels were considered elevated when ≥ 0.2 EU/mL; ^cOverall *p* value, the result of statistical comparison of placebo-treated subjects vs. all (pooled) subjects treated with eritoran tetrasodium. *P* values were derived from Fisher's exact test (gender, race, SIRS, SOFA, organ failure, endotoxin level, drotrecogin alfa [activated] therapy, corticosteroid therapy), Chi-square test (number of organ failures) or analysis of variance (age, height, weight, APACHE II score, IL-6).

to subjects who received high-dose (105 mg) eritoran tetrasodium, using Fisher's exact test. Bonferroni multiplicity adjustment required $\alpha \leq .008$ for statistical significance.

We also performed exploratory sensitivity analyses on the primary end point of 28-day mortality rate based on three variables: clinically evaluable subjects (CMH chi-square test), subjects with no use of DAA (CMH chi-square test), and survival distribution (Kaplan-Meier product limit and log-rank test).

Additional exploratory tests were planned to identify which of the following categorical

covariates might interact on the primary end point: APACHE II-predicted mortality rate (low or high), type of pathogen, age (≤ 65 or > 65 yrs), stage of study (I, II, or III), use of DAA during the study period, baseline endotoxin level (detectable or ≥ 0.2 EU/mL), baseline high-density lipoprotein level (< 25 or ≥ 25 mg/dL), time to drug infusion (< 8 or $8-12$ hrs), center by geographical location (United States or Canada), and center by size (small, medium, large). Construction of a multivariate model was planned for any covariates with statistical significance $p < .05$.

After conclusion of the study, it became known that unblinding information might have been included in shipment of study drug for up to 40 subjects at 14 study sites. However, pharmacy personnel at these study sites stated that unblinded information was not given to investigators and clinical personnel. Mortality analysis of the MITT population excluding these potentially unblinded patients produced results similar to those of the entire MITT population.

Statistical testing was performed with version 8.02 of the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS

Baseline Characteristics

A total of 300 subjects were randomly assigned to the three intervention groups. Seven subjects did not receive any study drug. The 293 MITT subjects (Fig. 1) were randomized as follows: 96 subjects to placebo, 103 subjects to eritoran tetrasodium 45 mg, and 94 subjects to eritoran tetrasodium 105 mg.

Baseline characteristics for eritoran tetrasodium- and placebo-treated subjects are shown in Table 1. There were no significant differences in baseline demographics, acute physiologic abnormalities, chronic health problems, and number of organ failures. Refractory shock was present at baseline in 76.8% of all subjects. Shock was the most common qualifying organ system failure (50.1% of all study subjects), followed by acute renal dysfunction and respiratory failure. The groups did not differ significantly in mean baseline APACHE II scores and had similar proportions of subjects with high APACHE II-predicted risk of mortality. Stress-dose corticosteroids were given by treating physicians in 27.6% of all subjects. Circulating endotoxin (≥ 0.01 EU/mL) was detected at baseline in $\geq 71\%$ of subjects from each group. Median baseline endotoxin levels were similar between groups, with a wide range of values within each of the three groups. Endotoxin values were higher in subjects with high APACHE II-predicted mortality rate. Baseline IL-6 values varied widely in the three groups but were not statistically different between treatment groups.

The most common focus of infection (Table 2) was the lung in all groups, followed by intra-abdominal and urinary tract infections. Culture results

Table 2. Characteristics of infection

Characteristic	Placebo (n = 96)	Eritoran Tetrasodium 45 mg (n = 103)	Eritoran Tetrasodium 105 mg (n = 94)	Overall <i>p</i> Value ^a
Primary focus of infection, n (%)				.5054
Pulmonary	38 (39.6)	39 (37.9)	29 (31.2)	
Intra-abdominal/gynecologic	20 (20.8)	14 (13.6)	15 (16.1)	
Urinary tract	13 (13.5)	13 (12.6)	18 (19.4)	
Skin/soft tissue	5 (5.2)	7 (6.8)	7 (7.5)	
Indwelling catheter	2 (2.1)	8 (7.8)	5 (5.4)	
Unknown	3 (3.1)	8 (7.8)	9 (9.7)	
Other	7 (7.2)	4 (3.9)	5 (5.4)	
No evidence of infection	8 (8.3)	10 (9.7)	5 (5.4)	
Infection type, n (%)				.6944
Gram-negative	26 (27.1)	23 (22.3)	29 (31.2)	
Gram-positive	30 (31.3)	38 (36.9)	29 (31.2)	
Mixed bacterial	10 (10.4)	7 (6.8)	13 (14.0)	
Fungal	1 (1.0)	4 (3.9)	1 (1.1)	
Viral	1 (1.0)	2 (1.9)	0 (0)	
Unknown	17 (17.7)	18 (17.5)	15 (16.1)	
Bacteremia, without focal infection, n (%)	3 (3.1)	10 (9.7)	8 (8.5)	
Bacteremia, with focal infection, n (%)	25 (26.1)	29 (28.1)	26 (27.7)	
Adequate antimicrobial therapy, n (%)	87 (91)	91 (88)	85 (90)	

^aOverall *p* value, the result of statistical comparison of placebo-treated subjects vs. all (pooled) subjects treated with eritoran tetrasodium, derived from Cochran-Mantel-Haenszel chi-square test.

Table 3. Treatment-emergent signs and symptoms occurring in >5% of patients in the 105-mg dose group

	Placebo (n = 96)%	Eritoran Tetrasodium 45 mg (n = 103)%	Eritoran Tetrasodium 105 mg (n = 94)%	<i>p</i> Value ^a Eritoran Tetrasodium vs. Placebo
Anemia	16.7	18.4	23.4	.3333
Thrombocytopenia	8.3	8.7	5.3	.4202
Atrial fibrillation	7.3	4.9	12.8	.1807
Bradycardia	5.2	3.9	9.6	.2248
Constipation	2.1	10.7	9.6	.0442
Diarrhea	8.3	9.7	11.7	.4466
Pneumonia	9.4	6.8	5.3	.2828
Sepsis	13.5	14.6	13.8	.9304
Urinary tract infection	15.6	8.7	17.0	.3863
Hyperglycemia	7.3	7.8	6.4	.8027
Hypoglycemia	5.2	3.9	7.4	.5151
Hypokalemia	13.5	12.6	8.5	.3218
Hypomagnesemia	9.4	10.7	7.4	.6672
Agitation	9.4	5.8	8.5	.7787
Insomnia	1.0	2.9	5.3	.0941
Acute renal failure	1.0	3.9	6.4	.0533
Pleural effusion	5.2	1.9	5.3	.9967
Pulmonary edema	5.2	5.8	5.3	.9838
Respiratory failure	14.6	6.8	8.5	.1626
Rash	5.2	8.7	9.6	.2547
Hypertension	4.2	9.7	7.4	.3686
Hypotension	11.5	7.8	6.4	.1715

^aOverall *p* value derived from Cochran-Mantel-Haenszel chi-square test.

demonstrated a higher rate of Gram-positive (34.2% overall) than Gram-negative (26.5% overall) infection. In all MITT subjects, bacteremia was identified in 101 subjects across all treatment groups

(34.5%), definite bacterial focal site of infection was identified in 66.9%, definite or possible fungal infections were identified in 1.0%, and no evidence of infection (or unlikely focal infection) was present in 8.5%.

Subject Disposition

Of the 293 subjects who received study drug, 195 (67%) completed the 28-day observation period. Ninety-eight subjects (33%) discontinued treatment before study completion because of AEs, including death (29.7%), consent withdrawal (1.4%), or other reasons (2.4%). Overall, 30.7% (n = 90) of all subjects died during the 28-day observation period, and 47.8% of the deaths occurred during the 6-day infusion period.

Safety

Drug Exposure. Four of the 293 MITT subjects received fewer than four doses of study drug (one placebo, two in the 45-mg group, one in the 105-mg group). All other subjects received at least four doses or died during the 6-day infusion period. Median plasma drug levels were 2206 ng/mL in the eritoran tetrasodium 45-mg group and 4338 ng/mL in the eritoran tetrasodium 105-mg group, which would have been sufficient to completely block amounts of endotoxin usually observed in patients with severe sepsis (3, 29).

Adverse Events. A total of 280 subjects (95.6% of all subjects) had at least one TEAE (Table 3). TEAEs did not prevent any subjects from receiving the full dose of study drug for 6 days. In the eritoran tetrasodium-treated subjects, anemia, diarrhea, insomnia, acute renal failure, and rash were observed more frequently than in placebo-treated subjects, although not at a statistically significantly higher rate.

SAEs occurred in 71.9% of placebo subjects, 95.1% of eritoran tetrasodium 45-mg subjects, and 74.5% of eritoran tetrasodium 105-mg subjects. SAEs that occurred in 5% of subjects in one or more treatment groups included cardiac arrest, multiorgan failure, sepsis, respiratory failure, and deep vein thrombosis. Four eritoran tetrasodium-treated subjects experienced a hepatobiliary SAE, compared with none in the placebo group. Three of the four subjects with hepatic SAEs had preexisting liver disease.

Atrial fibrillation occurred at a higher rate in the eritoran tetrasodium 105-mg group compared with the placebo group (*p* = .18). Instances of atrial fibrillation were mild or moderate and unrelated to study drug administration.

Phlebitis occurred in 3.0% of eritoran tetrasodium-treated groups and did not

Table 4. Infectious adverse events (%)

Infectious Adverse Events	Placebo (n = 96)	Eritoran Tetrasodium 45 mg (n = 103)	Eritoran Tetrasodium 105 mg (n = 94)	Overall <i>p</i> Value ^a
Investigator-reported infectious complication	50.5	45.6	37.0	.2001
Clinical evaluation committee determination of infectious complication ^b	36.8	35.0	38.0	.8967

^aOverall *p* value is based on the approximation of count; ^bFisher's exact test was used.

Infectious adverse events were defined as either a) recurrent infection at the same site as the sepsis-initiating infection, either relapse of the same organism, or superinfection by a different organism; or b) new infection occurring at a different site than the sepsis-initiating infection.

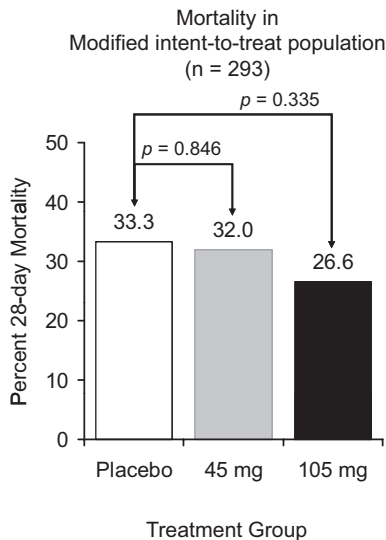


Figure 2. All-cause mortality rate at day 28 by treatment group. In the modified-intent-to-treat population, eritoran tetrasodium 105 mg did not result in a statistically significant decrease compared with placebo (Cochran-Mantel-Haenszel chi-square test, *p* = .335).

occur in the placebo-treated group (*p* = .21, chi-square). The frequency of phlebitis was 1.5% and 1.6% in the eritoran tetrasodium 45-mg and 105-mg treatment groups, respectively, when the drug was administered via a central venous catheter. The rate was higher (5.7% to 6.7%) among the 65 subjects who received one or more doses of eritoran tetrasodium through a peripheral venous catheter. Venous thrombosis occurred in 26 subjects (8.9%) and was independent of eritoran tetrasodium treatment status (10.4% placebo group, 10.7% eritoran tetrasodium 45-mg group, and 5.3% eritoran tetrasodium 105-mg group).

The occurrence of infectious complications (AEs) reported by study investigators did not differ significantly among groups (Table 4). Infectious AEs included worsening of sepsis, new infection, and

superinfection. The clinical evaluation committee found insufficient objective data for 55% of the investigator-reported infectious AEs.

Safety Laboratory Tests. The three groups had similar mean values of aspartate transaminase, 5'-nucleotidase, and leucine aminopeptidase throughout the 28-day study. Compared with placebo, the eritoran tetrasodium 105-mg group had more episodes of leukocytosis (*p* = .029), more episodes of elevated creatinine (*p* = .03), and more episodes of elevated alanine transaminase (*p* = .086). Autopsies, or postmortem liver tissue samples from 11 (12.2%) of the 90 subjects who died, showed no pathologic findings related to eritoran tetrasodium treatment.

Mortality Analyses

In the MITT population, all-cause 28-day mortality rate did not differ significantly between the subjects treated with eritoran tetrasodium 45 mg or 105 mg compared with placebo (Fig. 2). While the observed 28-day mortality rate in the MITT population was 6.7% lower in subjects treated with eritoran tetrasodium 105 mg compared with placebo, this observation was not statistically significant (*p* = .335).

Subgroup Analyses. The effect of treatment with the higher dose (105 mg) on 28-day all-cause mortality rate was further analyzed in prespecified subgroups (Fig. 3). Although lower mortality rates were observed in subjects treated with eritoran 105 mg compared with placebo, within APACHE II quartiles 2 and 4, the differences observed were not statistically significant. Mortality rates for subjects in APACHE II quartiles 1 and 3 were higher for the group that received eritoran tetrasodium 105 mg compared with placebo, but these differences also were not significant statistically. For the subjects in the lowest quartile of APACHE II scores (<21), mortality rate was higher in the eritoran tetrasodium 105-mg treated group (12.0% vs. 0.0% placebo, CMH chi-square test, *p* = .083).

In subgroups of the MITT population, based on the presence of shock or no shock at baseline, mortality rate was not significantly lower for subjects who received 105 mg of eritoran tetrasodium compared with placebo (Fig. 3b).

Sensitivity Analyses. We performed three predefined sensitivity analyses to investigate the primary end point. These analyses were intended to be exploratory

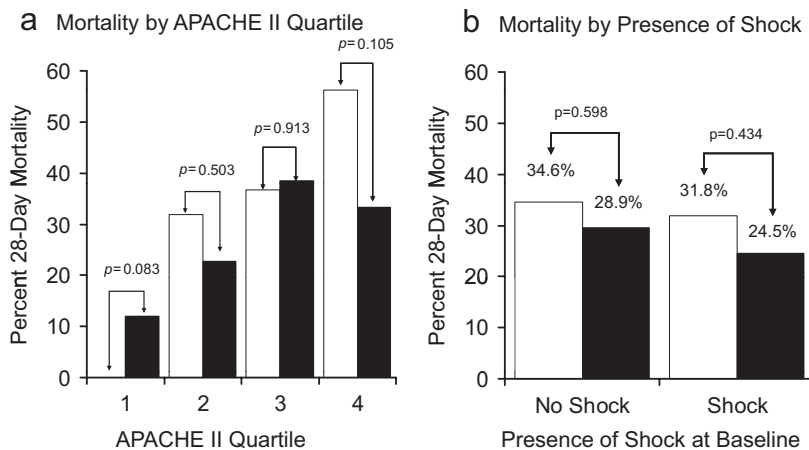


Figure 3. Prespecified subgroup analyses. Mortality rate in subjects treated with 105 mg of eritoran tetrasodium (*n* = 94, black bars) compared with placebo (*n* = 96, white bars). a, Effect of Acute Physiology and Chronic Health Evaluation (APACHE) II score quartile on 28-day all-cause mortality rate in modified intent-to-treat population. Quartile 1 corresponds to score <21 (105-mg subjects, *n* = 25; placebo, *n* = 23); quartile 2, score 21–24 (105-mg subjects, *n* = 22; placebo, *n* = 22); quartile 3, score 25–28 (105-mg subjects, *n* = 26; placebo, *n* = 19); quartile 4, score >28 (105-mg subjects *n* = 21; placebo *n* = 32). b, effect of presence or absence of shock on 28-day all-cause mortality rate in modified-intent-to-treat population.

only, and all observations of lower mortality rates were not statistically significant. First, in the population of clinically evaluable subjects (Fig. 4a and Methods), 28-day mortality rate was 12.5% lower in

the eritoran tetrasodium 105-mg group compared with placebo. Second, in the population that did not receive DAA, 28-day mortality rate was 6.9% lower in the eritoran tetrasodium 105-mg group com-

pared with placebo (Fig. 4b). Third, graphic representation of survival by Kaplan-Meier survival-time curves of the three treatment groups were analyzed by log-rank tests (Fig. 5a). There were no differences in survival time in the MITT population. Additional *post hoc* analyses of survival time in subgroups defined by predicted mortality rate calculated from APACHE II score (Fig. 5, b and c), or by APACHE II score ≥ 21 (not shown), suggested a trend toward lower mortality rate in subjects who received 105 mg of eritoran tetrasodium and had a higher risk of death at baseline that was not statistically significant.

Covariates of possible clinical importance were evaluated for effect on 28-day mortality rate (Fig. 6). Upon individual testing, none of the following prespecified variables demonstrated a significant effect in patients treated with eritoran tetrasodium 105 mg compared with placebo: APACHE II-predicted mortality risk, type of pathogen (Gram-negative, Gram-positive, mixed bacterial), or other baseline covariates (age, gender, study stage, use of DAA, elevated endotoxin level, low level of high-density lipopro-

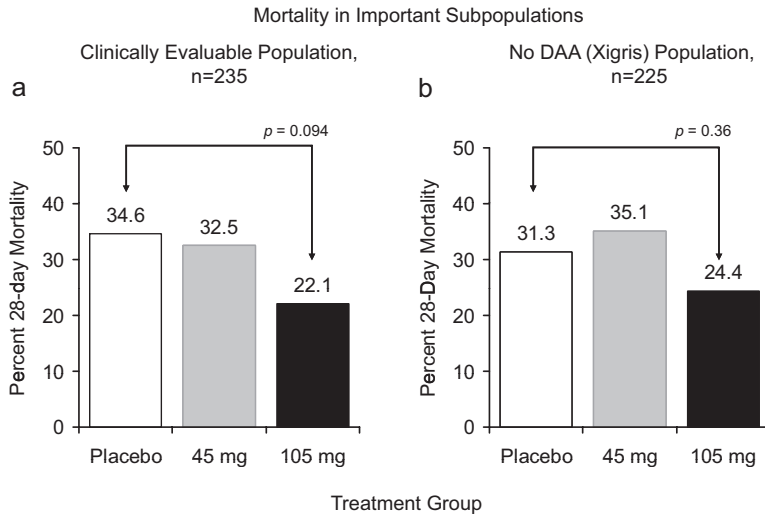


Figure 4. Additional sensitivity analyses. Twenty-eight day all-cause mortality rate by treatment group. *a*, the clinically evaluable population, $n = 235$. A mortality difference of 12.5% was observed between the 105-mg group (black bars) and placebo (white bars); exploratory analysis, $p = .094$. *b*, subgroup of subjects in whom drotrecogin alfa (activated) (DAA, Xigris, Eli Lilly & Co., Indianapolis, IN) was not used, $n = 225$. A mortality difference of 6.9% was observed between the 105-mg group ($n = 78$) vs. placebo ($n = 80$); exploratory analysis $p = .36$.

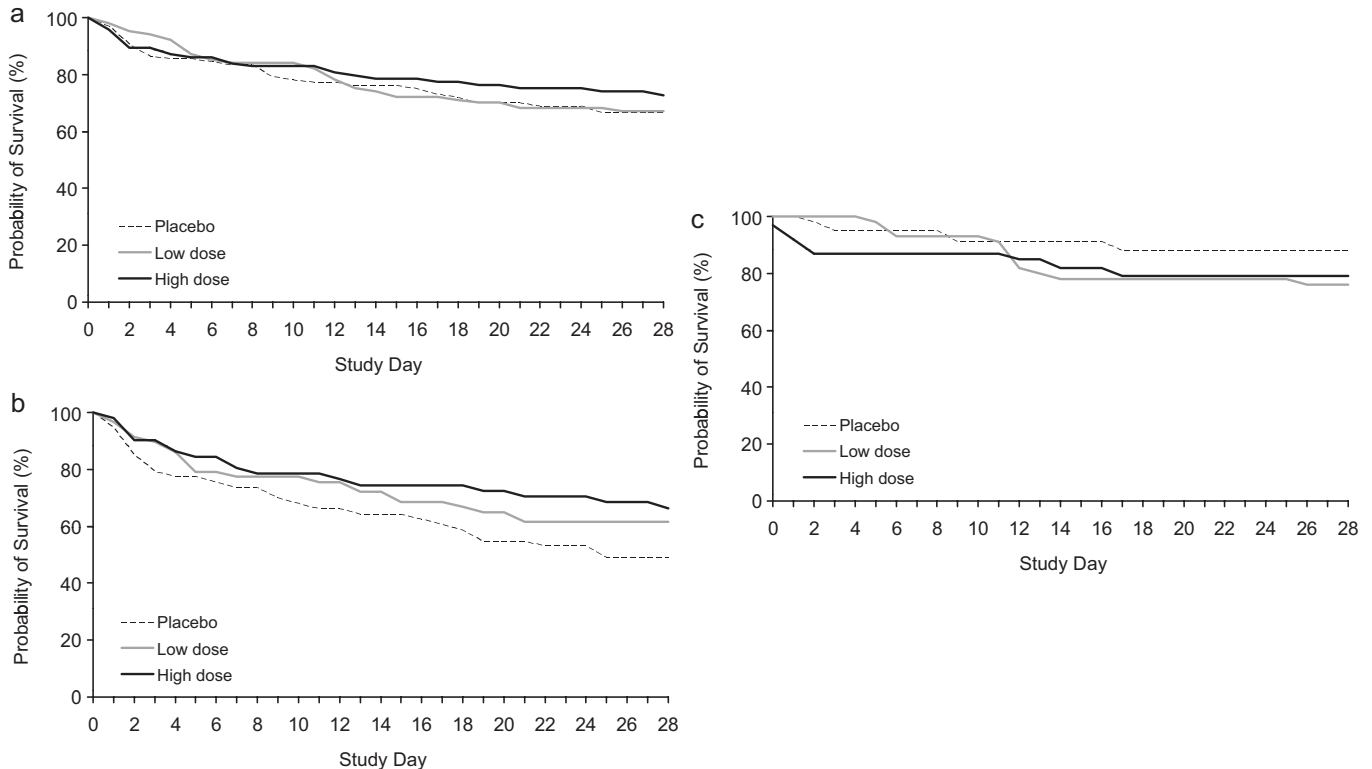


Figure 5. Kaplan-Meier survival-time curves through day 28. *a*, the modified intent-to-treat population consisting of placebo ($n = 96$), eritoran tetrasodium 45-mg ($n = 103$), or 105-mg ($n = 94$) groups. Survival in the eritoran tetrasodium 105-mg group was not statistically significantly greater than in the placebo group ($p = .366$). *b*, subgroup of subjects with high predicted risk of mortality (51% to 80%) by Acute Physiology and Chronic Health Evaluation (APACHE) II score: placebo ($n = 53$), eritoran tetrasodium 45-mg ($n = 58$), or 105-mg ($n = 51$) groups ($p = .0733$, 105 mg of eritoran tetrasodium vs. placebo). *c*, subgroup of subjects with low predicted risk of mortality (20% to 50%) by APACHE II score ($p = .314$, eritoran 105 mg vs. placebo).

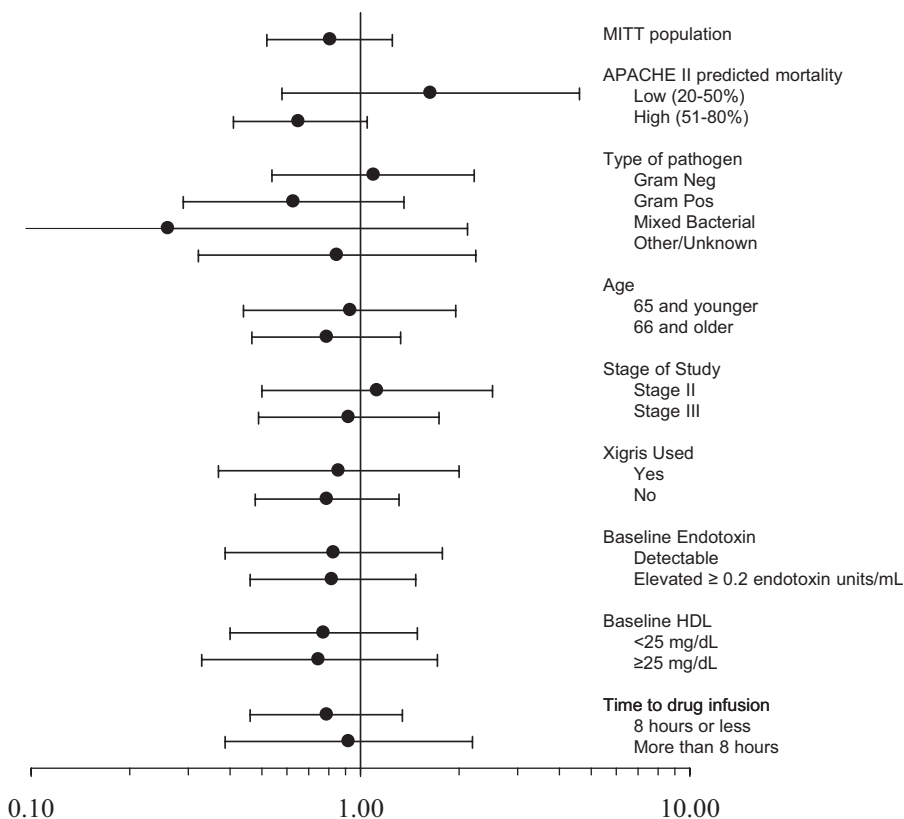


Figure 6. Relative reduction in risk of death at 28 days and 95% confidence interval of effect. Relative risk of death for patients who received eritoran tetrasodium 105 mg vs. placebo. An x-axis value of 1 signifies no effect. Values to the left of 1 indicate a relative risk reduction in favor of 105 mg of eritoran tetrasodium. The relative risk for the modified intent-to-treat (MITT) population is shown at the top. APACHE, Acute Physiology and Chronic Health Evaluation; HDL, high-density lipoprotein.

tein, hours from recognition of severe sepsis to randomization, or enrollment site). In addition, the site and type (focal, disseminated, and/or bacteremia) of infection had no detectable influence on 28-day mortality rate (Table 5).

Serum IL-6. Mean and median IL-6 values were elevated at baseline and were not significantly different among the three groups. There was a wide range of baseline values within each group. IL-6 baseline values decreased in each group at 12 and 48 hrs after study drug infusion (Fig. 7).

DISCUSSION

This phase II clinical study is the first to evaluate the safety and efficacy of eritoran tetrasodium in subjects with severe sepsis. Eritoran tetrasodium is a specific, competitive, small-molecule antagonist of activation of TLR4 by bacterial endotoxin. Eritoran tetrasodium dose regimen or placebo was initiated within 12 hrs of recognition of severe sepsis and repeated every 12 hrs for 6 days. Eritoran tetraso-

dium was generally well tolerated, with few TEAEs. In the MITT population, although mortality rate was 6.7% lower among subjects treated with eritoran tetrasodium 105 mg compared with placebo, this difference in mortality rate was not statistically significant. Additional planned analyses were done to define the target population for a future phase III trial. In subgroups of the MITT population, subjects at higher risk of death based on APACHE II score quartile, and subjects in shock at baseline, there was a not statistically significant trend toward lower mortality rate among subjects treated with 105 mg of eritoran tetrasodium compared with placebo. While mortality rate trended lower in exploratory analyses of clinically evaluable subjects treated with eritoran tetrasodium 105 mg, this observation was statistically significant. Mortality rates did not appear to be influenced by whether the infection was caused by Gram-negative organisms, Gram-positive organisms, or fungal sepsis.

There was no evidence that endotoxin levels (either within the normal range or

elevated) were associated with different mortality rates for the eritoran tetrasodium 105-mg group compared with the respective placebo group (Fig. 6).

Our findings notwithstanding, endotoxin is a major microbial mediator of septic shock, and this pathogen-associated microbial pattern molecule remains an attractive target for the treatment of sepsis for many reasons. Endotoxin in human and animal models produces rapid inflammatory and coagulopathic changes seen during sepsis, and in animals endotoxin can produce lethal multiorgan failure (26, 30, 31). Circulating endotoxin levels are frequently detectable in the bloodstream of intensive care unit patients, and in those with severe sepsis, high endotoxin levels are associated with increased mortality rate (1, 3).

In this trial, clinical criteria for severe sepsis identified subjects with endotoxemia. Endotoxin was detected in serum at baseline in 80% of subjects and exceeded the normal range in 55% of subjects. Consistent with prior reports (1, 3), endotoxin was present in the blood sera of subjects with infection caused by Gram-positive bacteria or fungi and not only Gram-negative bacterial infections. Mortality rates did not appear to be influenced by the type of infecting pathogen. There was no indication that subjects with differing sites of infection (pulmonary or intravascular) responded differently to the doses of eritoran tetrasodium administered, as suggested by recent rat experiments (32). As intended, the plasma levels of eritoran tetrasodium attained in subjects in this trial were similar to the levels observed in previous phase I pharmacokinetic trials, in which eritoran tetrasodium was administered to normal volunteers before endotoxin challenge and shown to inhibit signs and symptoms of endotoxemia (26).

Despite evidence for the role of endotoxin in producing sepsis syndrome, previous trials of other seemingly promising drugs targeting endotoxin in subjects with severe sepsis did not lead to lower mortality rates. Antibody-based approaches against endotoxin that used low-affinity and poly-specific immunoglobulin regimens with variable biological activity resulted in inconsistent clinical benefits. Although antiendotoxin antisera against the mutant *Escherichia coli* J5 initially appeared to improve outcome in septic shock (10), these favorable results could not be reproduced in a large phase III trial (33). Specific monoclonal

Table 5. Mortality by site of infection

Type of Infection	Placebo (n = 96)	Eritoran Tetrasodium 45 mg (n = 103)	Eritoran Tetrasodium 105 mg (n = 94)
Bacteremia only, n	3	10	8
28-day mortality rate (%)	33.3	40	50
Possible/definite focal only, n	59	51	54
28-day mortality rate (%)	37.3	33.3	22.2
Possible/definite focal plus bacteremia, n	25	29	26
28-day mortality rate (%)	24	27.6	30.8
Neither possible/definite focal nor bacteremia, n	9	13	6
28-day mortality rate (%)	33.3	30.8	16.7
Pulmonary, n	38	39	29
28-day mortality rate (%)	30.8	30.8	27.6
Intra-abdominal/gynecologic	20	14	15
28-day mortality rate (%)	55.0	35.7	20.0
Urinary tract, n	14	13	18
28-day mortality rate (%)	7.1	30.8	27.8
Skin and soft tissue	5	7	7
28-day mortality rate (%)	40.0	42.9	42.9
Other/unknown/no infection	20	30	23
28-day mortality rate (%)	31.6	30.0	20.8

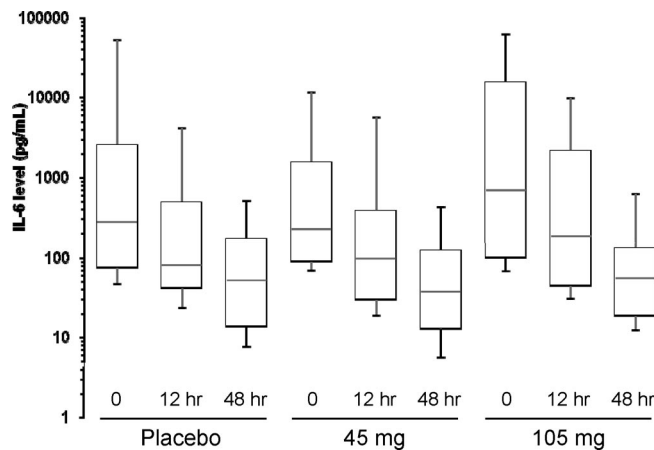


Figure 7. Values of serum interleukin (IL)-6 in each of the three treatment groups at baseline and at 12 and 48 hrs after start of administration of study drug grouped by treatment assignment for the modified intent-to-treat population (n = 293 at baseline). Box plots represent median, 10th, 25th, 75th, and 90th percentiles.

antibodies directed against the lipid A component of endotoxin (the monoclonal immunoglobulin M antibodies HA-1A and E5) also failed to demonstrate mortality benefit (16, 17, 19). Subsequent investigations indicated that these were low-affinity antibodies that only weakly bound and neutralized endotoxin (34). Another endotoxin antagonist is bacterial permeability-increasing protein (BPI), an antibacterial protein produced within the azurophilic granules of human neutrophils. A recombinant, 21-kD, truncated form of the N-terminal domain of human BPI (rBPI₂₁), which binds endotoxin with high affinity and efficiently neutralizes endotoxin activity, has been extensively

studied in human endotoxin challenge studies and clinical sepsis studies. This recombinant form of BPI improved some morbidity indices but did not significantly decrease the mortality rate in a phase III trial in children with meningococemia (13).

In contrast, eritoran tetrasodium differs structurally from endotoxin-blocking antibodies, antisera, and recombinant proteins used in previous studies. Eritoran tetrasodium is a small, modified lipid A antagonist generated by chemical synthesis that competes with the lipid A component for endotoxin from Gram-negative bacteria for binding to the TLR4-MD-2 complex (4). In addition, eri-

toran tetrasodium is functionally unique in its ability to interfere with the interaction of endotoxin with the TLR4-MD-2 transmembrane signaling complex found on the cell surface of endotoxin-responsive cells. Therefore, eritoran tetrasodium, in contrast to many previous therapeutic agents designed to interfere with endotoxin, has the ability to block the effects of endotoxin in a human volunteer model of endotoxemia (26).

We analyzed predefined covariates for possible effects of eritoran tetrasodium on mortality rate (Fig. 6). The inflammatory and coagulopathic effects of endotoxin are believed to play a key role in the pathogenesis of severe sepsis and are known to occur within hours. In this study, treatment with eritoran tetrasodium or placebo was started as soon as possible. Initially, the protocol called for a time window of 8 hrs from recognition of severe sepsis; however, the protocol was later amended to permit a window of ≤12 hrs. There was no statistical difference in outcome for the initiation of eritoran tetrasodium within 8 hrs, or 8–12 hrs, after identification of severe sepsis. A time window of 12 hrs for administration of eritoran tetrasodium appears to be both feasible and appropriate, although we could not determine precisely how long subjects had severe sepsis before diagnosis, because severe sepsis usually started before arrival at the hospital. We cannot rule out the possibility that a shorter window for administration may improve outcome, especially given the benefits observed with early administration of fluids and antibiotics in patients with severe sepsis (35).

Our results suggest that the APACHE II severity of illness score (and predicted mortality) is not only an indicator of 28-day mortality rate in study subjects but also could be a predictor of positive response to eritoran tetrasodium. For subjects in APACHE II quartile 1, eritoran tetrasodium appeared to have no or possibly a negative effect on mortality rate. These results are in line with the outcome of subjects with low APACHE II scores observed in the PROWESS (36, 37) and ADDRESS (38) trials. In these previous trials, placebo mortality rate was 12% to 17% among subjects with low severity of illness. Our finding of a placebo mortality rate of 0% in the subjects with the lowest APACHE II quartile scores (compared with 12% for subjects treated with 105 mg of eritoran tetrasodium) is unexpected and likely reflects small sample

size. However, we cannot entirely rule out the possibility that eritoran tetrasodium had a deleterious effect in septic subjects with a low predicted mortality risk. A meta-analysis of human clinical trials of anticytokine therapies suggested an effect only in subjects with the highest risk of death (39), raising the possibility that anticytokine and other therapies for severe sepsis block inflammation that could be beneficial in some subjects. Based on analyses of subgroups in this study, a phase III trial of eritoran tetrasodium will use the 105-mg dose of eritoran tetrasodium and limit enrollment to subjects with severe sepsis and a significant risk of mortality (APACHE II scores ≥ 21 , quartiles 2, 3, and 4).

As expected in subjects with severe sepsis, the frequency of AEs was high in all three study arms. There were no significant differences in SAES, TEAEs, or clinically significant laboratory values attributed to study drug. There was no increase in the rates of new infections in the subjects treated with eritoran tetrasodium. Endotoxin responsiveness via TLR4 is an important "alarm" mechanism that initiates a protective innate immune response against microbial invasion (40, 41), yet excess and persistent endotoxin levels can also provoke severe sepsis and shock. Although we did not directly assess whether eritoran tetrasodium adversely affected immune function, there were no indications that eritoran tetrasodium interfered with recovery from primary infection or increased secondary infections.

A small number of subjects in each group had hepatic failure or new renal failure requiring hemodialysis. Future studies with eritoran tetrasodium will include close monitoring of subjects with preexisting hepatic or renal disease and preferential administration of this intravenous agent via a central venous catheter to minimize the risk of phlebitis.

The principal limitation of this exploratory phase II trial is that the small number of subjects enrolled and the staged study design prevent definitive conclusions about the safety and efficacy effects of eritoran tetrasodium. This phase II trial was intended to assess dose and whether to proceed to a phase III trial. Calculation was not used to determine the sample size. Other limitations of this trial include many found in other comparable therapeutic trials for severe sepsis, including a heterogeneous population; variable time from the onset of severe sepsis to administration of study

drug; a wide range of severity of illness scores; differences in delivery of care among different institutions; and difficulties determining the level of supportive care, withdrawal of supportive measures, and appropriateness of medical care (42). These factors will be addressed in part by using a clinical evaluation committee again for subjects enrolled in the phase III trial.

The APACHE II score, although it is less likely than other scoring systems to accurately predict mortality, was selected for this trial to stratify randomization and to ensure that subjects were not at too low or too high a risk of death after discussion with the Food and Drug Administration, shortly after release of the results of the PROWESS study of drotrecogin alfa (activated). APACHE II is also nonproprietary and allows comparisons with the patient populations enrolled in sepsis trials during the last 20 yrs.

The *in vivo* biological activities of eritoran tetrasodium previously seen in phase I trials of normal volunteers were not observed in this phase II trial in the target population of patients with severe sepsis. Possibly, this was due to differences of patients, endotoxin source, timing of severe sepsis and endotoxemia, or other uncontrolled factors. Only one marker of inflammation due to severe sepsis, IL-6, was measured in this study. It may have been important to measure other markers. There was no change in IL-6 from baseline levels specific to the administration of 105 mg of eritoran tetrasodium (Fig. 7) when compared with placebo. Interpretation of the IL-6 levels and changes was limited by the large variation in values in all groups at all time points. While a significant change in IL-6 would have coincided with current concepts of the mechanism of action of eritoran tetrasodium, the precise role of IL-6, and myriad other inflammatory mediators seen in severe sepsis, has not been fully elucidated. Inflammatory markers can provide additional support for a phase 3 study (43). However, the intended end point of this therapy for severe sepsis is the patient-centered benefit of decreased mortality rate.

CONCLUSIONS

The dosing strategy used in this study achieved plasma levels of eritoran tetrasodium previously shown to block signs and symptoms of endotoxemia in healthy adults. There were no major safety concerns when eritoran tetrasodium at 45 mg or 105 mg was administered to sub-

jects early in the course of severe sepsis. Despite the known antagonism of the TLR4-MD-2 receptor by eritoran tetrasodium, there did not appear to be an adverse effect on immune protection. We consistently observed a favorable, but not statistically significant, trend toward a lower mortality rate in subjects who received eritoran tetrasodium 105 mg in several predefined subsets of the study population. A trend toward a higher mortality rate was noted for subjects with low risk of mortality (APACHE II scores < 21 , first quartile). These results support the pursuit of further clinical trials that are powered to demonstrate an outcome benefit in patients with a high risk of mortality. Due to potential therapeutic benefit, the dose of eritoran tetrasodium 105 mg was selected to be used in a larger, international phase III clinical trial of patients with severe sepsis.

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APPENDIX 1

Definition of Severe Sepsis (Ref. 28)

Severe sepsis was defined as evidence of newly developed respiratory failure, refractory shock, renal dysfunction, hepatic dysfunction, or metabolic acidosis, presumed to be due to infection, in combination with at least three of four of the following signs of systemic inflammatory response syndrome within the 12 hrs preceding recognition of organ dysfunction:

- Fever or hypothermia (>38°C or <36°C)

- Tachycardia (heart rate >90 beats/min)
- Tachypnea (respiratory rate >20 breaths/min while breathing spontaneously, or $\text{PaCO}_2 < 4.3$ kPa, or use of mechanical ventilation for an acute respiratory process)
- Leukocytosis ($\geq 12 \times 10^3/\text{mm}^3$) or leukopenia ($< 4 \times 10^3/\text{mm}^3$) or >10% immature forms

APPENDIX 2

Sepsis-Induced Organ Dysfunction

- Refractory shock: systolic blood pressure <90 mm Hg or a decrease in systolic pressure of >40 mm Hg in the absence of other causes of hypotension. The decrease in blood pressure did not respond adequately to fluid challenge of 500 mL over 20 mins and required the use of vasopressors (excluding dopamine <5 $\mu\text{g}/\text{kg}/\text{min}$) to maintain a systolic blood pressure of >90 mm Hg.
- Respiratory failure: for subjects without pneumonia or other preexisting lung disease, acute lung injury ($\text{PaO}_2/\text{FIO}_2 < 300$ and diffuse bilateral pulmonary infiltrates in the absence of elevated left atrial pressure-related

pulmonary edema). For subjects with pneumonia without shock, evidence of acute respiratory distress syndrome ($\text{PaO}_2/\text{FIO}_2 < 200$)

- Acute renal dysfunction: serum creatinine of ≥ 2 mg/dL and, if known, at least twice the value before sepsis, in the absence of primary renal disease
- Acute liver dysfunction: elevation of total bilirubin to >3 mg/dL and elevation of alanine transaminase or aspartate transaminase to at least three times the upper limit of normal secondary to acute hepatic dysfunction and not related to other entities such as preexisting liver disease, biliary obstruction, or acute hemolysis
- Acute metabolic acidosis: $\text{pH} \geq 7.30$, or a base deficit of ≥ 5.0 mmol/L, in association with a plasma lactate level 1.5 times the upper limit of normal

APPENDIX 3

Key Exclusion Criteria

Subjects with any of the following were excluded from study participation:

- Individuals who, in the opinion of their physician, were unlikely to regain pre-morbid health status, or individuals for

whom commitment to aggressive treatment was uncertain

- Cardiogenic or hypovolemic shock
- Acute third-degree burns involving $\geq 20\%$ of body surface area
- Receipt of a nonautologous organ transplant within the past year
- Chronic vegetative state
- Uncontrolled serious hemorrhage requiring transfusion of >2 units of blood/platelets in the previous 24 hrs (if bleeding stopped and subjects were still otherwise qualified, they were considered for enrollment)
- Unwillingness or inability to be fully evaluated for all follow-up visits
- Classification as “do not resuscitate” or “do not treat” (subjects could be enrolled if all other means of resuscitation, including intubation and vasopressors, were part of treatment options)
- Development of systemic inflammatory response syndrome and organ failure <36 hrs after trauma or surgery
- Predicted risk of mortality score of <20% or >80% after recognition of qualifying organ failure
- Planned or current use of drotrecogin alfa [activated] (recombinant human activated protein C, Xigris) for subjects with an Acute Physiology and Chronic Health Evaluation (APACHE) II-predicted risk of mortality of 20% to 50% (i.e., APACHE II score <25)