Safety, Pharmacokinetics, and Pharmacodynamics of E5564, a Lipid A Antagonist, during an Ascending Single-Dose Clinical Study

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*E5564, a structural analog of the lipid A portion of lipopolysaccharide (LPS), is a potent antagonist of the biochemical and physiologic effects of LPS in several in vitro and in vivo models and is currently under clinical development as a possible therapeutic for the treatment of sepsis and septic shock. The objectives of this study were to (1) assess the safety and tolerability of E5564 following a 30-minute intravenous (IV) infusion, (2) evaluate the pharmacokinetic profile of E5564, and (3) measure the ability of E5564 to block LPS stimulation ex vivo in blood taken from subjects up to 8 hours after ending the infusion. Healthy male volunteers (*n = *7/dose group) were randomly assigned to each of four dose levels (350, 1000, 2000, or 3500* μg*). Within each dose group, 5 subjects received drug and 2 received placebo. E5564 or matching placebo was administered by a 30-minute infusion, and blood samples were collected at predetermined time points. All doses of E5564 were demonstrated to be safe and well tolerated. E5564 plasma concentrations were determined using a validated LC/MS/MS method. The C_{max} and AUC of E5564 increased in a dose-proportional manner. E5564 pharma- cokinetics were characterized by a slow clearance (0.67-0.95 mL/h/kg), a small volume of distribution (41-54 mL/kg), and a relatively long elimination half-life (42-51 h). As measured in the ex vivo assay, E5564 inhibited LPS-induced tumor necrosis factor–α (TNF-α) in a dose-dependent manner, and at the higher doses (2 and 3.5 mg), antagonistic activity was measurable up to 8 hours postinfusion. E5564 lacked LPS-like agonist activity at doses up to 3.5 mg. Taken together, we believe that E5564 is a safe, potent antagonist of LPS in blood and will likely benefit patients in the treatment of LPS-related diseases.*

Keywords: *Sepsis; E5564; pharmacokinetics; healthy volunteers; ex vivo; lipopolysaccharide*

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An estimated 70,000 to 300,000 cases of sepsis occur each year.[1,2] While the cause of sepsis is not fully understood, it has been suggested that sepsis syndrome and its resultant sequelae are the immunologic and/or inflammatory consequences of a host response to stress.[3] Endotoxin, or the lipopolysaccharide (LPS) component of the cell wall of gram-negative bacteria, is believed to be associated with septic response.[4,5] LPS alone can induce inflammatory responses similar to that of whole gram-negative bacteria, making it a likely trigger of host inflammatory response,[6] triggering the release of cytokines and other cellular mediators from monocytes and macrophages. Too robust a response to pathogen components (even those from dying pathogens) can cause fever, shock, organ failure, and other life-threatening conditions.[4]

The lipid A portion of LPS is believed to be the primary portion responsible for the binding of LPS to receptors on various target cells and the resultant toxicity of LPS.[7] Studies with E5531, a first-generation endotoxin (lipid A) antagonist, demonstrated that such an antagonist is capable of blocking LPS in vitro and in animal models without LPS-like agonist activities.[8-10] E5564, α-D-Glucopyranose, 3-O-decyl-2-deoxy-6-O-[2-deoxy-3-O-[(3R)-3-methoxydecyl]-6-O-methyl-2-[(11Z)-1-

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oxo-11-octadecenyl)amino]-4-O-phosphono-β-D-glucopyranosyl]-2-[(1,3-dioxotetradecyl)amino]-,1-(dihydrogen phosphate), tetrasodium salt (Figure 1), is a second-generation LPS antagonist and structural analog of the lipid A portion of LPS from the nontoxic bacterium *Rhodobacter sphaeroides*. Like E5531, E5564 inhibits the biochemical and physiologic effects of LPS in several in vitro and in vivo models but is more potent than E5531.[11] In addition, E5564 is more water soluble than E5531, making it easier to purify and formulate.[12]

Additional experiments demonstrated that E5564 reduced the incidence of mortality in animal models of antibiotic-treated systemic infection. Furthermore, since E5564 exerts its beneficial effects by blocking the first step in the cellular activation initiated by LPS, E5564 has demonstrated the ability to block all the effects of endotoxin.[11] This is in contrast to other agents tested for the treatment of sepsis such as PAF antagonists, IL-1α receptor antagonists, and antibodies to tumor necrosis factor–α (TNF-α), all of which act at single sites further down the cascade and thus inhibit only a limited portion of the effects of LPS. In previous in vitro and in vivo studies, E5531 (as well as E5564) was found to completely block or ameliorate all the effects of LPS immediately upon addition to whole blood or administered in vivo, but its activity was rapidly lost as a consequence of its binding to certain lipoproteins in blood or plasma.[13,14] The primary objective of this study was to assess the safety and tolerability of E5564 in healthy male volunteers following a 30-minute intravenous (IV) infusion of E5564 at sequential ascending strengths. The secondary objectives were to evaluate the pharmacokinetic (PK) profile of E5564 and to measure the ability of E5564 to block LPS in blood after a 30-minute IV infusion. Finally, correlations between dose and plasma concentrations of E5564 and the ability of LPS to elicit TNF-α in an ex vivo assay were examined.

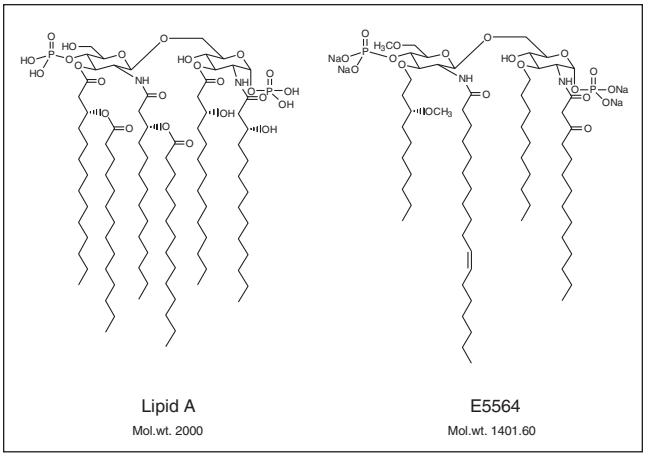

Figure 1. Chemical structures of E5564 and Escherichia coli *lipid A.*

METHODS

Study Population

Eligible subjects were healthy male volunteers between the ages of 18 and 45 years. Only subjects who weighed between 65 and 85 kg and were within 20% of their ideal body weight, as established by the Metropolitan Life Insurance Company tables, were eligible for study participation.[15] Concomitant medications were not permitted during the course of the study. The study protocol was approved by an institutional review board prior to the enrollment of the study subjects. All volunteers gave written informed consent prior to their participation in the study.

Study Design

This was a single-center, randomized, double-blind, placebo-controlled, sequential-group, single-dose study of E5564 in normal, healthy male volunteers. Subjects (7 per dose group) were assigned randomly to one of the four dose panels (350, 1000, 2000, or 3500 μg). Subjects within each dose group were further randomized such that 5 subjects received E5564 and 2 subjects received placebo. Subjects participated only in one dose group evaluation. Treatment safety and tolerability were evaluated for each dose group before dosing was initiated in the next higher dose group. E5564 (1000 μg per vial) or matching placebo was administered through a 30-minute infusion.

Subjects were monitored for safety and tolerability at regular intervals through 120 hours after administration of the dose. Safety and tolerability assessments included monitoring and questioning of the subjects about adverse events, physical examinations, clinical laboratory tests (including hematology, blood chemistry, and urinalysis), vital sign measurements (including supine and standing pulse rate and blood pressure), and 12-lead electrocardiograms (ECGs). Cytokine concentrations were also determined at 0.75, 1, 1.5, 2, 2.5, 3, and 4 hours to check whether E5564 exhibited any LPS-like agonistic activity.

Sample Collection and Analysis

E5564 Plasma Concentration Determination

Blood samples for determining plasma levels of E5564 were collected at predose (–1.0 h) and at 0.5 (immediately after the end of infusion), 0.6, 0.75, 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, 96, and 120 hours. E5564 concentrations in plasma were determined using a validated liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) method with a quantifiable limit of 5 ng/mL. Briefly, plasma (0.5 mL) was mixed with internal standard (IS, a structural analog of E5564) solution and then extracted with methanol (MeOH). The supernatant was collected and evaporated to dryness in a 50°C bath under a nitrogen stream, then reconstituted with MeOH. Each sample was analyzed on a Quattro LC/MS/MS system (Beverly, MA) using electrospray ionization under the negative-ion mode. E5564 was monitored at precursor ion m/z at 1312 and product ion m/z at 158.9, and the IS was monitored at precursor ion m/z at 1454.70 and product ion m/z at 159.0 in the negative-ion mode. The mobile phase was a mixture of 100% MeOH, 1% acetic acid, and 0.1% trifloroacetic acid (TFA). Aliquots were injected onto a Luna phenyl-hexyl (3 μm, 2 × 100 mm, Phenomenex, Torrance, CA) column. The retention times for IS and E5564 were 2.73 and 3.26 minutes, respectively. Standard curves for E5564 were linear over the concentration range of 5 to 1000 ng/mL with coefficients of variation less than 15%.

Analysis of E5564 Activity in Whole Blood

Heparinized blood samples were also collected at predose and 0.5, 0.75, 1, 2, 3, 4, 6, and 8 hours postinfusion, and 400 μL was dispensed to a 48-well cell culture cluster plate (Corning 3548). Then, 50 μL of 100 or 10 ng/mL LPS (final concentration: 10 or 1 ng/mL) in D5W and 50 μL of PBS were added. Vehicle (50 μL D5W) and 50 μL PBS were added to control nonstimulated samples. After a 3-hour incubation at 37°C/5% CO_2 with gentle shaking on a Belco plate shaker, all samples were reduced to plasma by centrifugation (900 × *g*) using an ELISA plate centrifuge. Aliquots (100 μL) were transferred into wells of duplicate 96-well microtiter plates and were analyzed for TNF-α by an R&D Quantikine ELISA assay kit. TNF-α was chosen as a marker for endotoxin activation because of its importance as a pro-inflammatory cytokine,[16] as well as its ability to be reproducibly induced and the ease of measurement by ELISA.

Pharmacokinetic Analysis

Pharmacokinetic parameters for E5564 were estimated using noncompartmental methods with the WinNonlin computer program, version 2.0 (Pharsight Corporation, Mountain View, CA). For each subject, the apparent maximum plasma concentrations (C_{max}) of E5564 and the corresponding times (t_{max}) were obtained by inspecting the data. The terminal rate constant, λ, was determined for each subject by linear regression analysis of the terminal linear portion of the semi-logarithmic plasma concentration versus time profiles of E5564. The terminal half-life, $t_{1/2}$, was calculated from λ as $t_{1/2} = 0.693/\lambda$.

The area under the plasma concentration versus time curve ($AUC_{0\text{-}t}$) from predose (0 h) until the last quantifiable concentration occurred (C_{last}) was calculated using the linear trapezoidal method. The area under the plasma concentration versus time curve from 0 hour to infinite time ($AUC_{0\text{-}\infty}$) was calculated as $AUC_{0\text{-}\infty} = AUC_{0\text{-}t} + C_{last}/\lambda$. The area under the first moment curve ($AUMC_{0\text{-}t}$) from predose (0 h) until the time t_{last}, at which the last quantifiable concentration of E5564 occurred in the plasma, was calculated using the linear trapezoidal rule. The area under the first moment curve of the E5564 concentration versus time curve from time 0 to infinity, $AUMC_{0\text{-}\infty}$, was calculated using the equation $AUMC_{0\text{-}\infty} = [(t_{last} \bullet C_{last})/\lambda] + (C_{last}/\lambda^2)$. The volume of distribution at steady state (Vdss) was calculated using the equation $Vdss = (Dose/AUC_{0\text{-}\infty}) \bullet (AUMC_{0\text{-}\infty}/AUC_{0\text{-}\infty})$.

Statistical Analysis

Analysis of variance (ANOVA) was used to test the randomization of demographic and baseline characteristics among the four treatment groups. All pharmacokinetic data are presented by dose group as mean ± standard deviation (*SD*). A nonparametric (Kruskal-Wallis test) method with Tukey's multiple-range test was used to examine effects of dose on E5564 PK parameters. The correlation between dose and PK parameters was determined using descriptive and nonparametric methods (Spearman test). The adverse events (AE) comparison between treatment groups was performed using Fisher's exact test. A *p*-value of < 0.05 was considered statistically significant.

RESULTS

Subjects

A total of 29 healthy male subjects completed the study. A subject in the 2000-μg group was inadver-

tently discontinued 5 minutes prior to receiving his complete (30-min) dose and was excluded from ex vivo (PD) and PK analyses. This subject was evaluated for safety of test drug only. A new subject was enrolled as his replacement. The average age of participants was 26.8 ± 1.3 years (range: 20-42), and the average weight was 78.5 ± 1.4 kg (range: 65.8-93.1). There was no statistical difference in body weight among the four treatment groups.

Adverse Events

No serious adverse events occurred, and there were no statistically significant differences in the incidence of treatment-emergent signs and symptoms in subjects who received E5564 compared to placebo. Four subjects treated with E5564 experienced headache: 1 in the 350-µg E5564 group, 2 in the 2000-µg E5564 group, and 1 in the 3500-µg E5564 group. One placebo-treated subject experienced injection site hemorrhage, while 2 E5564-treated subjects (1 in the 350-µg E5564 group and 1 in the 1000-µg E5564 group) experienced injection site reactions as well as injection site hemorrhage. Two subjects experienced pharyngitis: 1 in the 2000-µg E5564 group and 1 in the 3500-µg E5564 group.

Pharmacokinetics

Mean plasma concentration versus time curves of E5564 for subjects in each dose group are presented in Figure 2. Mean (± *SD*) of PK parameters are summarized in Table I. Plasma levels of E5564 increased slightly after the end of the 30-minute infusion, as reflected in the median t_{max}, which ranged from 0.6 to 0.75 hours. The t_{max} was independent of dose. Both C_{max} and $AUC_{0-\infty}$ increased in a dose-proportional manner with correlation of coefficients of > 0.96 and *p*-values of < 0.0001. The total clearance (CL) values were 0.95, 0.75, 0.67, and 0.71 mL/h/kg after 350, 1000, 2000, and 3500 µg, respectively, indicating that E5564 was cleared slowly from the body. The clearance (L/h/kg) was negatively correlated to the dose (µg/kg) with a correlation coefficient of –0.56313 and a *p*-value of 0.0097 (Figure 3). The clearance from the 350-µg group was significantly higher than that from the 1000-µg (p = 0.0351), 2000-µg (p = 0.0048), and 3500-µg (p = 0.0176) groups. However, there was no statistical difference among the 1000-µg, 2000-µg, and 3500-µg groups. The volume of distribution at steady state (Vdss) from subjects who received 350 µg (0.054 L/kg) of E5564 was significantly higher than that from subjects who received 1000 µg (0.041 L/kg, p = 0.0437). Similar to the clearance, there was no statistical difference in Vdss among the 1000-µg (0.041 L/kg), 2000-µg (0.047 L/kg), and 3500-µg (0.044 L/kg) groups. The small Vdss indicates that E5564 was not extensively distributed into tissues. The elimination half-lives ($t_{1/2}$) were 41.7 (350 µg), 42.4 (1000 µg), 50.5 (2000 µg), and 43.0 (3500 µg) hours and was independent of dose. The overall mean elimination $t_{1/2}$ was 44.4 hours and was similar to that obtained from dogs (50.4 h after a single IV of 0.3 mg/kg).

In conclusion, the C_{max} and $AUC_{0-\infty}$ of E5564 increased in a dose-proportional manner from 350 to 3500 µg after a 30-minute infusion of E5564 in male

Table I Mean ± *SD* Pharmacokinetic Parameters of E5564 in Healthy Male Volunteers following Administration of a Single IV Dose of E5564

	Group 1	Group 2	Group 3	Group 4
Dose (µg)	350	1000	2000	3500
Dose (µg/kg)	4.6 ± 0.3	12.9 ± 1.4	26.3 ± 3.3	48.0 ± 5.0
AUC_{0-120} (ng•h/mL)	4261.1 ± 561.4	15500.3 ± 3103.8	31700.3 ± 2198.3	57236.9 ± 6894.1
$AUC_{0-\infty}$ (ng•h/mL)	4924.4 ± 635.7	18030.0 ± 4196.6	39248.2 ± 3600.7	67870.9 ± 8418.2
t_{max} (h)[a]	0.75 (0.6-1)	0.75 (0.6-8)	0.75 (0.6-4)	0.6 (0.6-0.75)
C_{max} (ng/mL)	115.8 ± 25.5	365.9 ± 42.3	722.6 ± 81.9	1214.0 ± 195.3
$t_{1/2}$ (h)	41.7 ± 5.4	42.4 ± 6.3	50.5 ± 12.8	43.0 ± 7.6
CL (L/h)	0.072 ± 0.0098[b]	0.059 ± 0.018	0.051 ± 0.0047	0.052 ± 0.0066
CL (L/h/kg)	0.00095 ± 0.00011[b,c,d]	0.00075 ± 0.00017	0.00067 ± 0.000032	0.00071 ± 0.00011
Vdss (L)	4.12 ± 0.66	3.23 ± 0.30	3.57 ± 0.65	3.25 ± 0.52
Vdss (L/kg)	0.054 ± 0.0082[b]	0.041 ± 0.0027	0.047 ± 0.010	0.044 ± 0.0054

a. Values represent the median (range).
b. Significantly different from the 1000-µg group.
c. Significantly different from the 2000-µg group.
d. Significantly different from the 3500-µg group.

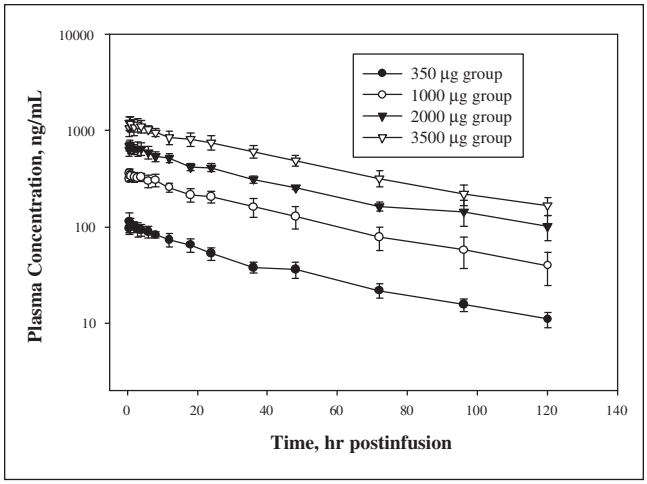

Figure 2. Semi-logarithmic plots of mean ± SD E5564 versus time in healthy male volunteers following a single IV dose of E5564. Blood samples from 5 volunteers each infused with 350 µg (●), 1000 µg (○), 2000 µg (▼), or 3500 µg (▽) E5564 were drawn prior to beginning infusion (–1 h) or at the indicated times after starting infusion and assayed for E5564 as described in the Methods and Materials section.

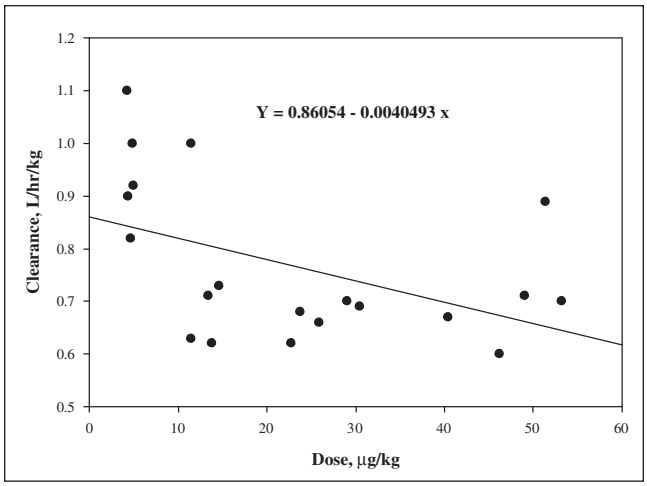

Figure 3. Correlation between clearance (L/h/kg) and dose administered (µg/kg).

healthy volunteers. The intersubject variation in PK parameters was relatively small, with coefficients of variation generally less than 20%. E5564 was characterized by a slow clearance (0.67-0.95 mL/h/kg), a small volume of distribution (41-54 mL/kg), and a relatively long half-life (42-51 h) in humans.

Inhibition of LPS-Induced TNF-α Release in Ex Vivo Blood Samples by IV Infusion of E5564

To obtain baseline values for LPS stimulation, we treated predose samples with 0, 1, or 10 ng/mL LPS as agonist. The reasons for choosing 1 and 10 ng/mL LPS for testing were that 1 ng/mL LPS was described as a clinically relevant concentration in patients with severe sepsis and septic shock,[5] and 10 ng/mL is believed to be an extremely high endotoxin concentration in any clinical condition. Blood from each subject generated TNF-α levels between 1204 and 6675 pg/mL (average = 4186 pg/mL) when 1 ng/mL LPS was used and between 1279 and 7393 pg/mL (average = 5050 pg/mL) when 10 ng/mL LPS was used as agonist. As shown in Figure 4, blood from volunteers who received placebo responded reproducibly to LPS throughout the analysis period. Compared to response values at predose (T_{-1} h), average response over the 8-hour period was 93.9% ± 8.5% for 1 ng/mL LPS and 97.1% ± 7.3% for stimulation by 10 ng/mL LPS. This indicated that response to LPS was reproducible with no measurable diurnal variation.

Figure 5A presents the response to 1 ng/mL of LPS-induced TNF-α release from samples taken from volunteers treated with E5564. Compared to the response at T_{-1} (before dosing), response was inhibited by 91.4% or more at the end of infusion of 350 µg. However, less than 50% of the activity was retained 1.5 hours later. This loss in activity was dose dependent. After infusion of 1000 µg E5564, approximately 36% inhibition was detected at 4 hours. The inhibitory activity 4 hours after the 2000-µg and 3500-µg doses increased to 63% and 75% inhibition of response, respectively. Subsequently, at 6 and 8 hours postinfusion, the lowest E5564 dose was inactive while the other doses showed trends, indicating that the activity was dose dependent. Figure 5B presents the average response to 10 ng/mL of LPS-induced TNF-α release from samples taken from volunteers treated with E5564. E5564 appeared to demonstrate less activity when higher doses of LPS were used to stimulate response. Compared to the response at T_{-1}, response at the end of infusion for each dose was 51% (350 µg E5564), 71% (1000 µg E5564), 90% (2000 µg E5564), and 95% (3500 µg E5564). Data suggested that more than 3500 µg of E5564 was needed to completely antagonize 10 ng/mL of LPS. Four hours after beginning the infusion of the lowest dose of E5564 (350 µg), antagonistic activity was below quantitation limit. In contrast, 45% of the inhibitory activity was retained for up to 8 hours in the highest (3500-µg) dose group.

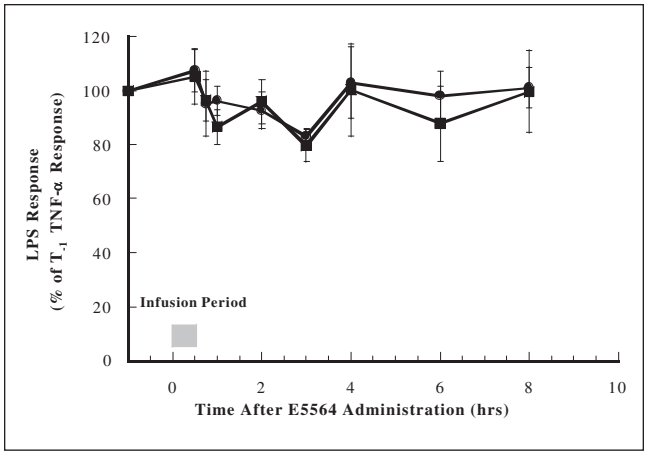

Figure 4. Induction of tumor necrosis factor–α (TNF-α) by lipopolysaccharide (LPS) in blood samples from placebo-treated volunteers for up to 8 hours after beginning infusion. Blood samples from 8 volunteers each infused with placebo were drawn prior to beginning infusion (–1 h) or at the times after starting infusion, as indicated on the x-axis, and were incubated in triplicate with 1 ng/mL LPS (■) or 10 ng/mL LPS (●) for 3 hours at 37°C. Plasma was assayed for release of TNF-α, as described in the Materials and Methods section. Percentage of T_{-1} response (y-axis) and standard error were calculated from the TNF-α release measured in blood taken 1 hour before drug administration.

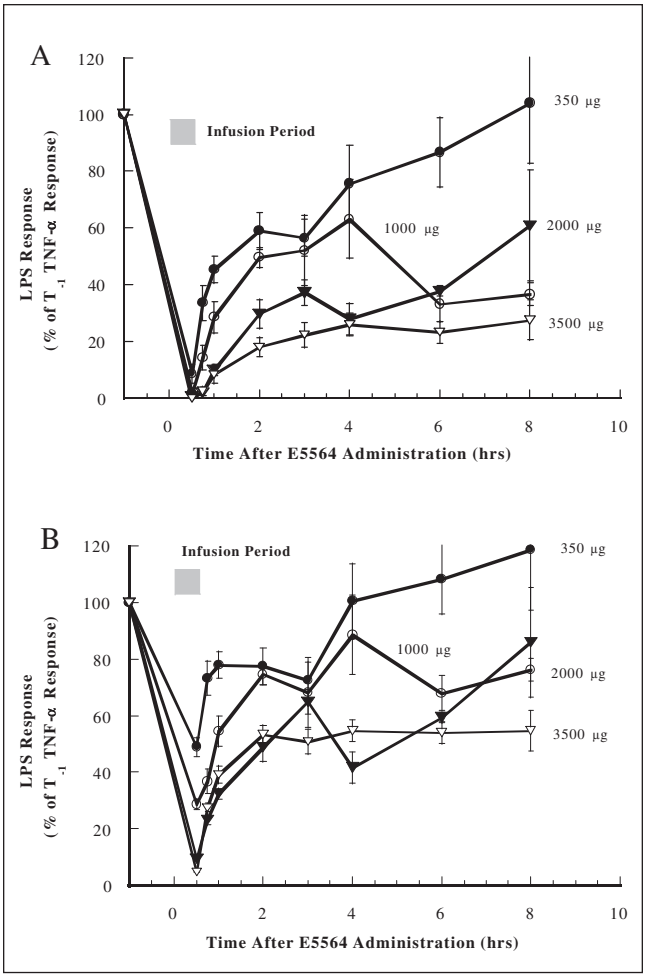

Figure 5. Average inhibition of lipopolysaccharide (LPS)–induced tumor necrosis factor–α (TNF-α) release in ex vivo blood samples from E5564-infused volunteers. Blood samples from 5 volunteers each infused with 350 µg (●), 1000 µg (○), 2000 µg (▼), or 3500 µg (▽) E5564 were drawn prior to beginning the infusion (–1 h) or at the times after starting the infusion, as indicated on the x-axis, and were incubated in triplicate with 1 ng/mL LPS (panel A) or 10 ng/mL LPS (panel B) for 3 hours at 37°C. Plasma was assayed for release of TNF-α, as described in the Materials and Methods section. Percentage of T_{-1} response (y-axis) and standard error were calculated from the TNF-α release measured in blood taken 1 hour before drug administration.

DISCUSSION

Endotoxin (LPS) has been associated with sepsis, a disease with a high mortality rate.[17] The administration of a small amount of LPS to healthy subjects produces a syndrome similar to that seen in clinical sepsis.[18,19] In a previously described placebo-controlled, double-blind study, healthy male volunteers received a 30-minute intravenous infusion of E5531, our first-generation lipid A antagonist, or placebo, and a bolus of LPS (4 ng/kg) was given to all subjects at the midpoint of infusion. The results indicated that in subjects receiving placebo, LPS caused headache, nausea, chills, and myalgia, whereas a dose-dependent decrease in these symptoms was observed in E5531-treated subjects, with as little as 250 µg completely blocking effects of LPS.[20] In addition, TNF-α and interleukin-6 (IL-6) blood levels were both lower in those who received E5531. In conclusion, E5531 blocks the symptoms and signs and cytokine and cardiovascular response seen in experimental endotoxemia. E5531 is a potent inhibitor of endotoxin response in humans, and it may be of benefit in the prevention or treatment of sepsis. In a recent in vivo model in dogs, infusion of E5564 blocked all significant changes (fever, vomiting, hypotension, tachycardia, neutropenia, elevated liver enzymes, and increased TNF-α and IL-6) induced by an LPS challenge,[21] indicating that E5564 retains its activity in vivo and may be effective against LPS-related diseases.

Single doses of E5564 from 0.35 to 3.5 mg given to normal male volunteers were demonstrated to be safe and yielded evidence of desired biological activities. A slow clearance (0.67-0.95 mL/h/kg), a small volume of distribution (41-54 mL/kg), and a long half-life (41.7-

50.5 h) were observed after a 30-minute IV infusion in humans. These parameters were similar to a synthetic analog of lipid A, ONO-4007,[22] which was in a clinical Phase I trial as an antitumor agent. ONO-4007 has a low systemic clearance (approximately 1.3 mL/min) and a small volume of distribution (5-8 liters) with a long half-life of 74 to 95 hours after a 30-minute IV infusion.

IV infusion of E5564 tested by ex vivo assay of peripheral blood demonstrates that PD and PK antagonistic activity of E5564 can be measured in normal volunteers. Dose dependence was observed for E5564 antagonism of LPS, and inhibition of higher doses of LPS required higher doses of E5564. This observation is consistent with our proposed mechanism of competitive antagonism. Inhibitory activity was greatest at the end of the infusion, with the highest circulating levels of E5564 in the body, but decreased much more rapidly than plasma level. The inhibitory activity rapidly decreased to immeasurable levels when the dose of E5564 was low (350 μg). At higher doses of E5564, the inhibitory activity was sustained up to 8 hours. In vitro research has suggested that activity in blood (or serum) is lost in a time-dependent fashion.[11] Other research done in vitro indicates that E5564 binds almost immediately to high-density lipoprotein (HDL) in serum (Wasan et al, submitted), and this binding results in its time-dependent inactivation that can be complete at low concentrations of E5564 (data not shown). However, association with HDL is not quantitative, with up to 40% associated with low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and the protein fraction of plasma. In this study, it is likely that the robust activity of E5564 observed at the end of infusion is due to "completely active" E5564 that may be associated with a variety of plasma components, and loss in this activity is due predominantly to loss of the HDL-bound fraction. E5564 that is also associated with one or more of the non-HDL subfractions may be responsible for the lower level of longer lasting activity. Further analysis of the different plasma fractions may reveal why a portion of antagonistic activity is retained after ending the infusion.

In conclusion, E5564 was demonstrated to be safe in healthy male subjects after receiving a 30-minute IV infusion of 0.35 to 3.5 mg. The C_{max} and AUC of E5564 increased in a dose-proportional manner. The intersubject variation in PK parameters was relatively small. PK of E5564 can be characterized by a slow clearance (0.67-0.95 mL/h/kg), a small volume of distribution (41-54 mL/kg), and a relatively long elimination half-life (42-51 h). As measured in an ex vivo assay, intravenously administered E5564 inhibited LPS-induced TNF-α in a dose-dependent manner. The antagonistic activity was measurable up to 8 hours postinfusion of higher doses (2 or 3.5 mg E5564). E5564 lacked LPS-like agonist activity at doses up to 3.5 mg. The observation that E5564 is safe and well tolerated indicates that administration regimens can be adjusted to attain longer lasting antagonistic activity, leading us to believe that E5564 can be used for the treatment of LPS-related diseases.

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