Extended in Vivo Pharmacodynamic Activity of E5564 in Normal Volunteers with Experimental Endotoxemia

Melvyn Lynn, Y. Nancy Wong, Janice L. Wheeler, Richard J. Kao, Carlos A. Perdomo, Robert Noveck, Ramon Vargas, Tony D’Angelo, Sandra Gotzkowsky, F. Gilbert McMahon, Kishor M. Wasan, and Daniel P. Rossignol

Eisai Medical Research Inc., Glenpointe Centre West, Teaneck, New Jersey (M.L., J.L.W., R.J.K., C.A.P., D.P.R.); Eisai Research Institute, Andover, Massachusetts (Y.N.W.); Clinical Research Center, New Orleans, Louisiana (R.N., R.V., T.D., S.G., F.G.M.); and The University of British Columbia, Vancouver, British Columbia, Canada (K.M.W.)

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ABSTRACT

E5564 is a synthetic antagonist of bacterial endotoxin that has been shown to completely block human endotoxin response. Low doses of E5564 (0.35–3.5 mg) have a long pharmacokinetic half-life, but a surprisingly short ex vivo and in vivo pharmacodynamic half-life (generally less than several hours). To determine whether extended antagonistic activity can be achieved in vivo, this study assesses the pharmacodynamic activity of 4- and 72-h infusions of E5564 into normal volunteers. Administration of 3.5 mg of E5564/h × 72 h completely blocked effects of endotoxin challenge at the end of dosing (72 h), and at 48 and 72 h postdosing. Similarly, a 4-h infusion of E5564, 3 mg/h completely blocked endotoxin administered 8 h postdosing. A lower dose of E5564, 0.5 mg/h × 4 h, ameliorated but did not block most effects of endotoxin 8 h postdosing (p < 0.05). Finally, the effect of varying plasma lipoprotein content on E5564 activity was studied in subjects having high or low cholesterol levels (>180 or <140 mg/dl) after 72-h infusion of 252 mg of E5564. No differences were observed. These results demonstrate that E5564 blocks the effects of endotoxin in a human model of clinical sepsis and indicate its potential in the treatment and/or prevention of clinical sepsis.

Sepsis remains the most common cause of death in intensive care units in the United States, with a current estimate of >750,000 cases per year, and 215,000 deaths annually (Bone et al., 1989; Parrillo et al., 1990; Bone, 1994; Angus et al., 2001). Endotoxin (lipopolysaccharide from Gram negative bacteria, or LPS) has been implicated as a major cause of this syndrome (Suffredini et al., 1989a,b; Parrillo et al., 1990; Danner et al., 1991; Rodrick et al., 1992; Taveira da Silva et al., 1993; Opal et al., 1999). E5564 is a synthetic second generation analog of the lipid A component of LPS (Rossignol et al., 1999) that has demonstrated potent anti-LPS activity in a variety of in vitro and in vivo model systems (Mullarkey et al., 2003). In vitro analyses of the antagonistic activity of E5564 after infusion into healthy volunteers determined that even though E5564 has a long pharmacokinetic half-life and metabolism of E5564 is not observed, E5564 demonstrates a relatively short pharmacodynamic (PD) half-life (Wong et al., 2003). In vivo research further supports this observation by demonstrating that low doses of E5564 are extremely active when coadministered with LPS in the human endotoxemia model (Lynn et al., 2003) but rapidly loses antagonistic activity (Rossignol and Lynn, 2003), likely due to its interaction with plasma lipoproteins (Wasan et al., 2003). Similar loss in PD activity has been observed in a dog endotoxin infusion model (Suganuma et al., 2000).

Recently, we have established that higher doses of E5564 (36–252 mg) given as 72-h intravenous (i.v.) infusions are safe and well tolerated, except for the incidence of phlebitis (Rossignol and Lynn, 2003). In this same study, pharmacokinetic analysis of the disposition of E5564 determined that similar results found in vitro, E5564 was found predominantly associated with lipoproteins. However, blood samples taken from subjects infused with E5564 were hyporesponsive to endotoxin for up to 72 h after completion of infusion.

ABBREVIATIONS: E5564, α-0-glucopyranose, 3-O-decyl-2-deoxy-6-O-[2-deoxy-3-O-[(3R)-3-methoxydecyl]-6-O-methyl-2-[[112]-1-oxo-11-oc-tadecenyl]aminor]-4-O-phosphono-β-0-glucopyranosyl]-2-[[13-dioxotetradecyl]amino]-1-(dihydrogen phosphate), tetrasodium salt; CAS Registry No. 185954-98-7; LPS, lipopolysaccharide; PD, pharmacodynamic; PK, pharmacokinetic; HDL, high-density lipoprotein; AE, adverse event; WBC, white blood cell; ANOVA, analysis of variance; AUC, area under the curve; bpm, beats per minute; TNF, tumor necrosis factor; IL, interleukin.
To support our observation that extended antagonistic activity may be achieved in vivo, we have assessed response of subjects to a small dose of LPS (4 ng/kg) at one of three time periods: at the end of infusion, and after 48 and 72 h after ending infusion.

In addition, because HDL inactivates both E5564 and endotoxin, we have assessed the ability of E5564 to antagonize endotoxin activity in subjects having low-to-normal cholesterol levels (total cholesterol < 140 mg/dl) and high-to-normal cholesterol levels (total cholesterol > 180 mg/dl) infused with 252 mg of E5564 and challenged with endotoxin at the end of infusion and at 48 h postinfusion.

Finally, endotoxin challenge was performed in volunteers 8 h after administration of either 2 or 12 mg of E5564 infused over 4 h.

Results indicate that compared to the low doses tested in previous studies, higher doses of E5564 (12–252 mg) demonstrate extended PD activity by blocking the effects of LPS in a human model of clinical sepsis. Dosing of E5564 by continuous infusion or intermittent dosing may be safe and effective.

**Materials and Methods**

**Subjects.** Male subjects who were 18 to 45 years old with normal physical exams and laboratory test results entered the study. These subjects were admitted to the clinical unit the evening before dosing and remained confined for the duration of the study. Subjects assigned either to blinded placebo or E5564 received similar i.v. infusions. All subjects received 4 ng/kg intravenous dose of LPS at the indicated time. Informed consent was obtained from all subjects and human experimentation guidelines of the U.S. Department of Health and Human Services were followed in the conduct of this clinical research.

Each treatment group consisted of eight subjects, randomized in two blocks of four blinded treatment assignments, including six E5564- and two placebo-treated subjects. For groups 1 and 2 only, four of the subjects enrolled in each group had screening total cholesterol values of > 180 mg/dl, whereas the other four had values of < 140 mg/dl. The four subjects (three infused with E5564) in group 3 were all high cholesterol, at which point we believed efficacy had been established and further study of the 72-h infusion was discontinued. Volunteers were enrolled in subsequent groups without regard to total cholesterol values if they met other criteria. No subject was enrolled more than once in this study. Two subjects were discontinued prematurely due to phlebitis and replaced.

**Drug Administration and in Vivo LPS Challenge.** Details on study procedures, including endotoxin preparation, drug/placebo reconstitution, and administration (by peripheral i.v. line) have been described previously (Lynn et al., 2003). At the indicated time, an intravenous injection of 4 mg/kg of purified LPS (40 EU/kg) was administered over a 1-min period.

The indicated number of subjects was treated with E5564 and LPS as follows: group 1, eight subjects (two subjects were discontinued due to phlebitis), 3.5 mg/h × 72 h (252 mg), LPS administered at the end of drug infusion; group 2, six subjects, 3.5 mg/h × 72 h (252 mg), LPS administered 48 h after the end of drug infusion; group 3, three subjects, 3.5 mg/h × 72 h (252 mg), LPS administered 72 h after the end of drug infusion, one subject in this group received placebo inadvertently from hours 22 to 24, a second (placebo) subject received 7 mg of E5564 from hours 22 to 24 (results from this subject are discussed separately); group 4, six subjects, 3 mg/h × 4 h (12 mg), LPS administered 8 h after the end of drug infusion; and group 5, six subjects, 0.5 mg/h × 4 h (2 mg), LPS administered 8 h after the end of drug infusion.

**In Vivo Response to LPS.** Volunteers remained awake, in an upright position (≥45 degree angle from supine) and continued to fast from food and fluids (other than water) for 2 to 4 h before and 5 h after LPS administration.

**Monitoring of Vital Signs and Sample Collection.** Temperature and pulse rate were recorded 1 h before study drug and every 8 h after the start of study drug infusion until LPS challenge. Vital signs were also recorded at 1 h before LPS challenge and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 16, and 24 h after LPS challenge. Beginning at 1 h before LPS challenge, lead II ECG were monitored continuously and semirecumbent blood pressure measured at least every 30 min for at least 5-h postchallenge and recorded at the same times as other vital signs. Follow-up physical examinations were conducted, and 12-lead ECGs were recorded 24 h after LPS challenge.

**LPS-Induced Adverse Events.** Administration of LPS to normal volunteers has been reported to induce a syndrome characterized by signs and symptoms, including chills, fever, tachycardia, myalgia, headache, nausea, and vomiting that occur and resolve within 12 h of receiving 4 mg/kg LPS (Michie et al., 1988; Revhaug et al., 1988; Suffredini et al., 1989a,b; Parrillo et al., 1990; Rodrick et al., 1992; Parrillo, 1993; Hochstein et al., 1994; von der Mohlen et al., 1995). Occurrence of these and other adverse events were collected by regular elicitation and spontaneous complaints. These events were characterized as to severity (mild, moderate, and severe) and duration. Based on the adverse events (AEs) expected to result from LPS and temporal relationship with LPS challenge, the investigator determined whether an AE was LPS-related, E5564–related, or not related to either.

**Analysis.** Cytokines, white blood cells (WBCs) with differential, C-reactive protein, and clinical chemistries were assayed using standard validated procedures. Absolute lymphocyte counts were calculated as the total WBC percentage of lymphocytes and absolute neutrophil count as the total WBC percentage of neutrophils + bands + immature forms. Mean peak (or nadir for blood pressure and absolute lymphocytes) values for vital signs, C-reactive protein, total WBC counts, absolute lymphocyte and neutrophil counts, and cytokines are reported as change from respective baselines. LPS-induced adverse events are reported by incidence. Pharmacokinetics and distribution of E5564 into plasma lipoproteins was performed as described (Wasan et al., 2003; Wong et al., 2003). Samples for distribution into lipoprotein were available for five high cholesterol and three low cholesterol subjects.

**Statistical Analysis.** Analysis of variance (ANOVA) was used to test for differences between means of multiple groups. Dunn’s test was applied to assess differences between E5564 groups and placebo. Adverse events were compared among treatment groups by Fisher’s exact test. Events were only counted if they occurred for the first time or worsened after the start of drug administration. If a subject experienced the same event at multiple times, the event was counted once in the analysis using the greatest severity.

The five treatment groups were comparable with regard to race, age, weight, and height, surgical history, ECG, and physical examination.

Two subjects were removed from the study prematurely. One subject in group 1 received the complete E5564 regimen of 3.5 mg/h × 72 h but did not receive LPS due to E5564-associated phlebitis and WBC elevation. Similar adverse event occurred in the replacement subject. A second replacement subject completed the regimen and received LPS. Phlebitis was considered to be related to study drug infusion and resolved over the next few days in both discontinued subjects. As a result of these findings, the concentration of infusion solution of E5564/placebo was reduced, and the infusion site was moved as soon as irritation was observed. These changes of procedures dramatically reduced the incidence and severity of infu-
tion site irritation. No phlebitis was reported in groups 4 and 5, indicating that the duration of the infusion (72 h in groups 1, 2, and 3 versus 4 h in groups 4 and 5) was related to the development and severity of phlebitis.

Results

Safety

Phlebitis was associated with 72-h continuous i.v. administration of E5564 but not with 4-h infusions of E5564 into a peripheral vein. No other AEIs were associated with E5564 administration.

Pharmacokinetics

Drug concentrations were measured up to a maximum of 72 h after completion of 4- or 72-h infusion in this study. This provided too short a period of time to measure the elimination half-life, so calculations were done for the following three parameters: peak concentration (C<sub>max</sub>), time to reach maximum concentration dependent on the time of the infusion (T<sub>max</sub>), and area under the plasma concentration curve.

The results of these analyses are shown in Table 1. T<sub>max</sub> occurs at the end of the infusion or shortly thereafter. C<sub>max</sub> and AUC<sub>0-last</sub> were related directly to the dose administered. Groups 1, 2, and 3 had different AUC<sub>0-last</sub> because of different measurement periods. However, results were consistent with those obtain in our first 72-h continuous infusion study (D. P. Rossignol, manuscript in preparation).

Pharmacodynamic Activity

Analysis of Low- and High-Cholesterol Subgroups.

For groups 1 to 3, in which subjects were stratified by total cholesterol values at baseline (≥140 and ≥180 mg/dl), there were no apparent differences in laboratory or physiological responses to LPS (i.e., subjects randomized to receive placebo had a full response to LPS), and none of the subjects who received E5564 responded to LPS, regardless of whether subjects had low or high total cholesterol. In addition, no statistically significant differences were found in the distribution of E5564 in plasma lipoproteins determined at the end of infusion (72 h). As shown in Table 2, more than 60% of E5564 recovered from plasma was found in the HDL fraction, and distribution into the lipoprotein fractions was not significantly different between the low- and high-cholesterol groups. For these reasons, results for groups 1 and 2 are presented as the summary data combined from the low- and high-cholesterol subgroups. Stratification of subjects by baseline cholesterol level was discontinued for groups 4 and 5.

Clinical Syndrome Induced by LPS. Expected LPS-induced events occurred in subjects who received placebo and LPS. These include chills, fever, tachycardia, nausea, headache, myalgia, leukopenia followed by leukocytosis, and increased plasma cytokines and C-reactive protein and mild decreases in blood pressure, and alterations in WBC counts (Michie et al., 1988; Revhaug et al., 1988; Suffredini et al., 1989a,b; Rodrick et al., 1992; Parrillo, 1993; Hochstein et al., 1994; Lynn et al., 2003). LPS-induced alterations in body temperature and heart rate and symptoms resolved within 12 h after dosing and other signs resolved somewhat later. No clinically relevant or significant adverse effects observed, other than phlebitis, were judged to be associated with administration of E5564.

Vital Signs. All but one of the subjects who received placebo and LPS demonstrated a clinically significant rise in body temperature (≥38°C). As shown in Table 3, compared with placebo, E5564 inhibited this increase in a dose-dependent manner. E5564, 3.5 mg/h × 72 h effectively blocked the fever response whether LPS was administered at the end of the infusion, at 48 h postinfusion or at 72-h postinfusion (groups 1, 2, and 3, respectively; p < 0.01). Similarly, E5564, 3 mg/h × 4 h blocked the fever response when LPS was administered at 8 h postinfusion (p < 0.01). The 2-mg dose (0.5 mg/h × 4 h) of E5564 ameliorated this response in a statistically significant manner (p < 0.01); however, the degree of amelioration was less than the other E5564 groups.

LPS-Induced Tachycardia. Subjects who received placebo and LPS demonstrated a clinically significant rise in heart rate, with a mean maximum increase of 35 bpm at approximately 4 to 5 h after LPS challenge. As shown in Table 3, E5564, 3.5 mg/h × 72 h effectively blocked tachycardia whether LPS was administered at the end of the infusion, at 48 h postinfusion or 72 h postinfusion (groups 1, 2, and 3, respectively; p < 0.05 compared with placebo). Similarly, E5564, 3 mg/h × 4 h blocked tachycardia when LPS was administered 8 h after infusion (p < 0.01). Whereas the E5564, 0.5 mg/h × 4 h regimen ameliorated this response in a statistically significant manner (p < 0.05), the degree of amelioration was less than the other E5564 groups.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Pharmacokinetic parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion</td>
<td>252 mg (3.5 mg/h × 72 h)</td>
</tr>
<tr>
<td>Group number (n)</td>
<td>1 (n=6)</td>
</tr>
<tr>
<td>Time of LPS challenge (hours</td>
<td>0</td>
</tr>
<tr>
<td>postdose)</td>
<td></td>
</tr>
<tr>
<td>Mean plasma [E5564] in µg/ml at</td>
<td>40.9 (6.9)</td>
</tr>
<tr>
<td>time of LPS challenge (S.D.)</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt; mean in</td>
<td>2790.14² (478.0)</td>
</tr>
<tr>
<td>µg/ml · h (S.D.)</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; mean in µg/ml</td>
<td>45.5 (9.6)</td>
</tr>
<tr>
<td>(S.D.)</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; median in hours</td>
<td>73 (72–76)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data presented is combined for the low- and high-cholesterol subgroups of groups 1 and 2.

¹ The AUC is dependent on the duration of the study (AUC<sub>0-last</sub>), calculated by trapezoidal rule AUC<sub>0–36</sub>.

² AUC<sub>0–144</sub>.

³ AUC<sub>0–168</sub>.

⁴ AUC<sub>0–368</sub>.
Physiological and biochemical response to endotoxin

Lipoprotein distribution of E5564 in high- and low-cholesterol subjects

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Total Cholesterol</th>
<th>E5564 in HDL</th>
<th>E5564 in LDL</th>
<th>E5564 in VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low cholesterol (n = 3)</td>
<td>137.3 (2.7)</td>
<td>67 (4.5)</td>
<td>16.3 (6.6)</td>
<td>3.3 (0.88)</td>
</tr>
<tr>
<td>High cholesterol (n = 5)</td>
<td>180.6 (7.6)</td>
<td>68.6 (4.9)</td>
<td>15 (5.6)</td>
<td>2.8 (0.49)</td>
</tr>
<tr>
<td>Statistical significance</td>
<td>0.003</td>
<td>0.81</td>
<td>0.88</td>
<td>0.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Mean (S.E.)</th>
<th>E5564, 3.5 mg/h × 72-h Time Postdose to LPS Challenge</th>
<th>E5564, 3 mg/h × 4 h, LPS 8-h Postdose</th>
<th>E5564, 0.5 mg/h × 4 h, LPS 8-h Postdose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group No.</td>
<td></td>
<td>0 h</td>
<td>48 h</td>
<td>72 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital signs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (Δ, °C)</td>
<td>2.11 (0.28)</td>
<td>0.50 (0.11)**</td>
<td>0.33 (0.05)**</td>
<td>0.39 (0.11)**</td>
</tr>
<tr>
<td>WBC</td>
<td>113 (1.4)</td>
<td>1.9 (0.4)**</td>
<td>0.6 (0.3)**</td>
<td>1.5 (0.4)**</td>
</tr>
<tr>
<td>C-reactive protein (Δ, mg/dl)</td>
<td>4.2 (0.8)</td>
<td>0.3 (0.1)**</td>
<td>-0.2 (0.1)**</td>
<td>0.0 (0.0)**</td>
</tr>
<tr>
<td>TNFα</td>
<td>751 (338)</td>
<td>0 (0)**</td>
<td>0 (0)**</td>
<td>0 (0)**</td>
</tr>
<tr>
<td>IL-6</td>
<td>2945 (1235)</td>
<td>33 (13)**</td>
<td>13 (6)**</td>
<td>9 (3)**</td>
</tr>
</tbody>
</table>

*Compared to placebo group, p < 0.05; **compared to placebo group, p < 0.01.
* Number of subjects in each group are described in Table 1.

LPS-Induced Decreases in Blood Pressure. In this study, subjects who received placebo and LPS demonstrated mild decreases in systolic and diastolic blood pressure with the mean maximum decreases of -15.1 and -8.7 mm Hg, respectively, occurring at approximately 5 to 12 h after LPS challenge. Only one subject (in the placebo group) exhibited clinical hypotension (systolic blood pressure <90). As shown in Tables 3 and 4, the changes in blood pressure in the E5564 groups were similarly small and were not statistically different from placebo.

Changes in C-Reactive Protein. All subjects who received placebo and LPS demonstrated a clinically significant rise in C-reactive protein at approximately 24 h post-LPS. In groups 1 and 2, some subjects who received E5564 for 72 h experienced phlebitis syndrome by the end of infusion, including rises in C-reactive protein. For these two groups, before LPS challenge, mean C-reactive protein levels were 0.672 and 0.457 mg/dl, respectively. For other groups, the baseline C-reactive protein levels ranged from 0.00 to 0.111 mg/dl. As shown in Table 3, E5564 inhibited the LPS-induced increase in C-reactive protein in a statistically significant manner. Inhibition of this response was complete (100%) for groups 2 and 3 (72-h infusions) and nearly complete (98%) for group 4 (12-mg infusion/LPS at 12 h). Group 1 showed incomplete inhibition, probably due to the phlebitis syndrome-induced rise in C-reactive protein. Low-dose E5564 (group 5; 2 mg infusion/LPS at 12 h) ameliorated the C-reactive protein response by only 70%, even though cytokines were blocked by >97%.

Changes in WBCs. After an initial modest decline within 3 h after LPS injection, the WBC rises sharply, showing a maximal increase in WBC at approximately 12 h post-LPS. The initial decline is characterized as a transient neutropenia and a more prolonged lymphopenia, followed by a clinically significant neutrophilia. As shown in Table 3, E5564 inhibited this increase in WBCs in a statistically significant manner (p < 0.01). Subjects who received E5564 in groups 1 to 4 (252- and 12-mg groups) demonstrated small changes in WBCs, absolute neutrophils and absolute lymphocytes compared with placebo. Subjects who received the 2-mg dose of E5564 in group 5 demonstrated amelioration of these effects; however, complete blockade of changes was not achieved.

LPS-Induced Adverse Events. LPS induces certain expected AEs. The most commonly reported symptoms of endotoxemia are chills, fever, myalgia, headache, nausea, vomiting, and tachycardia. There were statistically significant treatment effects (p < 0.05) in E5564-mediated inhibition of chills, fever, headache, tachycardia, nausea, vomiting and myalgia. As shown in Table 4, E5564 inhibited these AEs in a highly statistically significant manner (p < 0.01 for incidence of any LPS-induced AEs). Subjects in groups 1 to 4 who received E5564 demonstrated virtually complete blockade of all LPS-induced treatment-emergent signs and symptoms. Although subjects in group 5, who received 2 mg of E5564, demonstrated a decreased incidence or severity of the events when challenged with LPS 8 h postdose, reduction was statistically significant (p < 0.05) but incomplete (generally reduced by 50% or more).
LPS-Induced Cytokines. For subjects who received placebo/H11001 LPS, TNF/H9251 and IL-6 increased, reaching peaks at 1.5 and 4 h, respectively, after LPS injection. As shown in Table 3, E5564 blocked this response in a dose-dependent, statistically significant manner. In groups 1 to 3, the E5564, 3.5 mg/h × 72-h regimen blocked TNFα and IL-6 by 100 and 98.9 to 99.7% (p < 0.01), respectively. Similarly, 12 mg of E5564 (3 mg/h × 4 h) blocked TNFα and IL-6 by 100 and 99.6% or more, respectively (p < 0.01). Although the 2-mg dose of E5564 (0.5 mg/h × 4 h) ameliorated this response in a statistically significant manner (>98%, p < 0.01 for TNFα and >97%, p < 0.05 for IL-6), the degree of amelioration was somewhat less than the other E5564 groups.

Inadvertent Administration of E5564

One placebo subject inadvertently received a 7-mg infusion of E5564 (2 h infusion of E5564, 3.5 mg/h at 22–24 h of the 72-h infusion). At 144 h (120 h after ending this short infusion), this subject was challenged with LPS. This subject had a maximum increase in heart rate of 31 bpm, a maximum increase in body temperature of 1.67°C, a maximum increases in WBCs of 7.2 × 10^9/l and absolute neutrophils of 7.55 × 10^9/l, as well as a maximum decrease of 1.11 × 10^9/l in absolute lymphocyte count, and maximum increase in C-reactive protein of 4.13 mg/dl. The subject experienced moderate chills, headache, and myalgia, as well as mild fever. Comparing these responses to those described for placebo/LPS-administered subjects in Tables 3 and 4, indicates that 7 mg of E5564, administered 5 days previously (plasma level of E5564 of 959 ng of E5564/ml at the time of LPS administration) had little inhibitory effect on in vivo endotoxemia.

Discussion

Our first human pharmacokinetic analysis of E5564 indicated that it has a low volume of distribution and relatively long half-life (~40 h). This would suggest that daily dosing of E5564 should be sufficient for 24-h protection. However, ex vivo analyses of low dose infusion of E5564 previously established that even though E5564 was extremely active when first infused, it rapidly lost antagonistic activity (Wong et al., 2003). In the endotoxin challenge model, the minimum effective dose to completely block effects of LPS was determined to be 100 μg of E5564 if coadministered with LPS (plasma levels...
of E5564 were approximately 38 ng/ml). In contrast, incomplete block of endotoxin response was observed after administration of 4 times the minimum effective dose (400 μg) if administered 8 h before LPS administration (Lynn et al., 2003; Rossignol and Lynn, 2003). Similar lack of activity was found in one volunteer administered 7 mg of E5564 5 days before LPS challenge, even though plasma levels of E5564 were near 1000 ng/ml. This result suggests that low concentrations of E5564 are rapidly inactivated while in circulation (Rossignol and Lynn, 2003). Using our criteria of in vivo efficacy as a 100% ablation of response leaves us unable to determine whether partial E5564 activity remained; instead, it can be noted that in our more quantitative ex vivo assays from our first phase I study, some residual inhibitory activity could be observed at up to 8 h after infusion of higher doses of E5564 (up to 3.5 mg) (Wong et al., 2003).

How long must the pharmacodynamic activity of E5564 persist in vivo for it to be an effective therapeutic agent in severe sepsis?

Studies of patients with severe sepsis indicate that endotoxicemia may be detected during a few days after the first organ failure in severe sepsis (Opal et al., 1999). This suggests the need to block LPS activity for about a week to provide a sufficient time window of benefit. Clearly, to provide protection for this extended period of time, either continuous low-dose infusion or intermittent dosing is required. This study evaluates the in vivo activity of 72 h infusion and lower dose, shorter 4-h infusions.

In a separate study, we have established that higher doses of E5564 (36–252 mg) given as 72-h infusions are safe and well tolerated, except for the incidence of phlebitis (Rossignol and Lynn, 2003). In addition, ex vivo analysis indicated that blood from treated subjects was hyperresponsive to LPS for at least 72 h after ending infusion. To support the possibility that we had achieved long-term antagonistic activity, we have assessed response of subjects to a small dose of LPS (4 ng/kg) at the end of infusion, 48 h after and 72 h after ending the 72-h infusion. In this study, all subjects who received placebo and endotoxin (4 ng/kg) experienced a mild transient syndrome similar to the initial signs of clinical sepsis. All subjects who received 3.5 mg of E5564/h × 72 h demonstrated complete blockade of LPS response (signs, symptoms, cytokines, C-reactive protein, and adverse events) at the end of dosing (72 h) and 48 and 72 h postdosing. This result indicates that at least a portion of the E5564 infused into blood retains its activity for at least 72 h after administration.

The substantial levels of E5564 in blood at the end of infusion are likely to be far more than is needed to block endotoxin response against even a very high challenge dose of endotoxin. This drove us to next question whether indeed lower plasma concentrations of E5564 could retain sufficient activity to block a delayed endotoxin challenge. Our ex vivo and delayed endotoxin response results from the 72-h infusion study indicated that a plasma level of 11 μg/ml or less was sufficient to block response to the relatively high concentration of 1 ng/ml LPS ex vivo. To begin determination of a minimum level, we estimated that one-third of this plasma level could be readily achieved through a single 4-h infusion of 12 mg of E5564 (3 mg/h × 4 h). Delayed endotoxin challenge of group 4 proved that this plasma level of E5564 was sufficient to protect against the effects of endotoxemia for 8 or more hours after ending infusion. Further lowering this dose by 6-fold resulted in plasma levels of 0.8 μg/ml and incomplete inhibition of endotoxin challenge. These results lead us to believe that E5564 can provide continuous protection against endotoxin when administered on a twice-daily dosing regimen establishing a steady-state plasma level of 3 μg/ml or more. In addition, because distribution of E5564 seems to be confined to the blood volume and plasma levels that exceed those necessary for optimum activity can be safely achieved, adjustment of dosing to body weight appears to be unnecessary.

We have previously shown that loss in antagonistic activity can be observed in vitro in human whole blood (Mullarkey et al., 2003) and is likely due to its interaction with plasma lipoproteins (Rose et al., 2000; Wasan et al., 2003). However, higher concentrations of E5564 (≥1 μM) are not quantitatively inactivated in vitro by whole blood, even after overnight incubation (data not shown), indicating that inactivation of E5564 is either time-dependent or is a “saturable” process. On the other hand, it is likely that as E5564 accumulates in the blood during longer infusions, higher concentrations of E5564 accumulate in plasma fractions that do not reduce its antagonistic activity. It is unclear as to which of these processes plays a role in permitting E5564 to retain activity in blood over an extended period of time.

Because lipoproteins inactivate both E5564 and endotoxin, we have assessed the ability of E5564 to antagonize endotoxin activity in subjects having low-normal cholesterol levels (total cholesterol <140 mg/dl) and high-normal cholesterol levels (total cholesterol >180 mg/dl) infused with 252 mg of E5564 and challenged with endotoxin at the end of infusion and at 48 h postinfusion. The differences in cholesterol found in our healthy volunteers had no effect on LPS response or on the activity of E5564 over this time period. Although septic patients have even lower levels of cholesterol (mean of ∼100 mg/dl) (Gordon et al., 2001), our observations suggest that E5564 will block LPS even when HDL levels are low.

In summary, we conclude that plasma levels of E5564 correlate to in vivo pharmacodynamic activity over extended periods of time (8–72 h) after ending infusion. In addition, although the 3.5 mg/h × 72-h infusion induced phlebitis syndrome in almost all subjects, 4-h infusions with E5564, 3 or 0.5 mg/h, had no apparent safety issues.

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References


Address correspondence to: Dr. Melvyn Lynn, Eisai Medical Research Inc., Glenpointe Centre West, 500 Frank W. Burr Blvd., Teaneck, NJ 07666-6741.

E-mail: melvyn_lynn@eisai.com