Pharmacokinetic-Pharmacodynamic Modeling of the Immunomodulating Agent Susalimod and Experimentally Induced Tumor Necrosis Factor-\(\alpha\) Levels in the Mouse

PETER GOZZI, INGRID PÅHLMAN, LENA PALMÉR, ALVAR GRÖNBERG, and STEFAN PERSSON

Department of Drug Metabolism Research, Pharmacia & Upjohn AB, Stockholm, Sweden

Accepted for publication June 14, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

The main objective of this study was to explore the concentration-effect relationship between the immunomodulating agent susalimod and lipopolysaccharide (LPS)-induced elevated serum levels of the proinflammatory cytokine tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)). Bacterial LPS (1 mg/kg) was given i.p. along with different doses of susalimod (0, 25, 50, 100, and 200 mg/kg) to female CD-1 mice. Blood samples were drawn at different time points (15–300 min), and serum was analyzed with respect to susalimod and TNF-\(\alpha\). The concentration-effect relationship was explored by modeling the data from all dose levels simultaneously using specially written program models, i.e., a three-compartment pharmacokinetic model, including biliary excretion, and an indirect mechanistically based pharmacodynamic model. The models, which were successfully fitted to the experimental data, showed that LPS induced the TNF-\(\alpha\) synthesis during ~70 min and that during this time course, the synthesis rate was governed by the serum pharmacokinetics of susalimod. Because the results supported the assumption that the maximum inhibitory effect was equal to full inhibition of the synthesis, the in vivo potency (\(IC_{50}\)) of susalimod could be estimated to 293 \(\mu M\). In conclusion, susalimod decreased the LPS-induced TNF-\(\alpha\) mouse serum levels in a concentration-related manner. The compound is suggested to inhibit the synthesis of TNF-\(\alpha\). The integrated pharmacokinetic-pharmacodynamic model estimated the in vivo potency of susalimod in the mouse to be 293 \(\mu M\).

The current knowledge about the mechanism of action of disease-modifying antirheumatic drugs (DMARDs) involves inhibition of the production of proinflammatory cytokines (Bondeson, 1997). In particular, tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) has been demonstrated to be of pivotal importance in rheumatoid arthritis (RA). The pharmacological effect of anti-inflammatory and immunomodulating agents is commonly studied with bacterial lipopolysaccharide (LPS) (Remick et al., 1989; Shapira et al., 1996) because administration of LPS into experimental animals leads to a rapid induction of macrophage-monocyte TNF-\(\alpha\) synthesis and develops similar pathophysiological changes to those of an inflammatory response. Hence, the effect of DMARDs on LPS-induced elevated TNF-\(\alpha\) exposure serves as a useful pharmacodynamic (PD) model for these types of agents.

In this study, we investigated the pharmacological action of susalimod, a metabolically stable chemical analog of sulfasalazine designed within a drug development program for the treatment of RA. The pharmacokinetics (PK) of susalimod has been investigated in various animal species (Påhlman et al., 1998). Its PK profile is characterized by an extensive biliary excretion, mainly as unchanged parent drug. Furthermore, the compound is highly bound to plasma albumin and has a small volume of distribution. In contrast to previously reported studies, which to our knowledge have only sought to describe a dose-response relationship, we have tried to establish a concentration-effect relationship between a compound (susalimod) and cytokine serum levels.

Integrated PK-PD modeling deals with the issue of correlating the time course of pharmacological effect intensity to the plasma pharmacokinetics of a drug. With its potential use in comparison of in vivo potency and in prediction of outcomes, PK-PD modeling is recognized to be of critical importance in the knowledge-gathering process of drug development (Yacobi et al., 1993; Breimer and Danhof, 1997). The possibility to perform relevant PK-PD modeling (i.e., in which adequate response variables are chosen and the rate-limiting steps are accurately identified) is very much depen-
dent on the knowledge about the molecular biology of drug action. In the common case where the pharmacological mechanism of action has not been fully clarified, modeling may nevertheless be viewed as a useful tool in exploring these events (Sheiner et al., 1979; Holford and Sheiner, 1981; Boxtel et al., 1992; Dayneka et al., 1993; Jusko and Ko, 1994; Danhof and Mandema, 1995).

In this study, serum concentration-time data of the immunomodulating agent susalimod and cytokine TNF-α were explored following concomitant administration of LPS and four different doses of susalimod to mice. In addition to TNF-α, other cytokines that are known to play a role in the biological response to LPS, e.g., the proinflammatory cytokine interleukin (IL)-6 as well as the anti-inflammatory cytokine IL-10, were determined. The effect of susalimod on these cytokines was found to be most evident for TNF-α. The concentration-effect relationship between susalimod and TNF-α was described by a specially designed integrated PK-PD model, comprising a three-compartment PK model and a mechanistically based indirect PD model.

Materials and Methods

Chemicals

Bacterial LPS (Escherichia coli; 0127-h8), batch 106923JE, was provided by Difco (Detroit, MI). LPS was used as a solution in 0.9% NaCl, 50 μg/ml. Susalimod, 2-hydroxy-5-[4-[[3-methyl-2-pyridinyl]sulfonyl]-phenyl]ethynyl]benzoic acid, mol wt 408 g/mol, was provided by Pharmacia & Upjohn (Upssala, Sweden). Susalimod was used as a solution in 0.9% NaCl (adjusted to pH 8.0) at concentrations of 1.25, 2.5, 5, or 10 mg/ml. Other chemicals used (see below) were of analytical grade and were obtained commercially.

Experimental Design

Two hundred female CD-1 mice, provided by Charles River, Germany, were used. The experiment was approved (C330/95) by the Animal Ethics Committee of Uppsala, Sweden. The mice were ~10 weeks old and weighed 20 to 25 g. A sublethal endotoxin shock was induced in all mice by administration of LPS, 1 mg/kg. Four groups received susalimod (25, 50, 100, or 200 mg/kg) in connection with the LPS administration, and one control group was given saline to explore the induced cytokine exposure in the absence of susalimod. The test articles were administered i.p., 0.02 ml/g b.wt., and blood samples were drawn from orbital plexus under anesthesia (Metofane; Mallinckrodt Inc., St. Louis, MO) at 15, 30, 45, 60, 90, 120, 180, and 300 min after administration of the test articles. Blood was collected from five mice at each time point. Each animal was sampled once and then sacrificed by neck dislocation. Serum was prepared and analyzed with respect to susalimod and TNF-α.

PK-PD Modeling

PK Model. The absorption, distribution, metabolism, and excretion characteristics of susalimod have been thoroughly investigated in various animal species, especially rat, dog, and monkey, but also mouse, rabbit, and mini-pig (Påhlin et al., 1998). In these studies, it has been shown that susalimod is mainly cleared via the bile without prior biotransformation, that clearance decreases with increasing dose, that plasma protein binding is very high, and that its volume of distribution is small. Furthermore, it has been shown that after intravenous administration of susalimod, the drug is excreted mainly in the bile with a small amount appearing in the urine. The drug is mainly cleared through enterohepatic recirculation as separate parameters, it was

\[
\frac{dC_L}{dt} = \frac{F\text{Dose}e^{-k_{\text{app}}t}}{V_c} + \frac{CL_{\text{renal}}C}{V_c} + \frac{CL_{\text{bile}}C}{V_c} + \frac{CL_{\text{cl}}C}{V_c} + \frac{CL_{\text{ext}}C}{V_c} + \frac{k_{\text{app}}A_{\text{ext}}}{V_c}
\]
The maximum inhibitory effect, \( I_{\max} \), was set to 1, i.e., the synthesis of TNF-\(\alpha\) was assumed to be fully inhibited at high enough susalimod levels. \( IC_{50} \) denotes the potency of susalimod, i.e., the serum concentration that produces 50% of \( I_{\max} \). A slope factor, \( n \), also was included in the Hill equation.

Modeling was performed by WinNonlin v 1.1 and the differential equations shown in eqs. 1 to 6. The PK model was first fit to the susalimod serum data and then the PK-PD model was fit to the TNF-\(\alpha\) serum data with the estimated PK parameters used as constants. In both cases, a simultaneous fit to all individual data from all dose groups was performed. The duration of synthesis, \( t_{\text{syn}} \), was first included as a parameter and then used as a constant in a final run. Goodness-of-fit of the nonlinear regression analysis was judged by the precision of the obtained parameter estimates, by potential parameter correlations, and by indications of any systemic deviations in the residuals (Gabrielsson and Weiner, 1997).

**Statistical Analysis**

The maximum observed serum levels of TNF-\(\alpha\) after LPS and susalimod administration were compared with the \( C_{\text{max}} \) obtained in the control group using the one-sided \( t \) test (Microsoft Excel 7.0).

**Results**

**Pharmacokinetics of Susalimod.** The proposed three-compartment PK model was fitted to the observed serum data of susalimod (Table 1; Fig. 2). Maximum serum levels of susalimod were observed at 15 (first sampling time point) or 30 min after dose administration. The model showed an extensive biliary excretion with a maximum bile clearance of 76 ml/min·kg. Bile clearance appeared to be saturated already at relatively low susalimod concentrations (\( K_m \) = 70 \( \mu \)M). Hence, during the first hour after the 25-mg/kg dose ~30% of the dose was estimated to reside in the gut, whereas the corresponding values after the 200-mg/kg dose was only between 6 and 7%. The estimated volume of distribution of the serum and tissue compartments was of the same magnitude, 0.26 and 0.37 l/kg, respectively. In the latter, slowly equilibrating compartment, peak concentrations of susalimod were estimated to be reached between 120 and 180 min.

**Pharmacodynamics and PK-PD.** Experimental data indicated that coadministration of susalimod and LPS leads to a dose-dependent reduction in the TNF-\(\alpha\) exposure with respect to both \( C_{\text{max}} \) and area under the serum concentration-time curve (AUC) (Table 2; Fig. 3). The interindividual variability in cytokine serum levels was very large with coefficient of variations generally in the order of 65% (in the treatment groups as well as the control group). A statistically significant decrease in \( C_{\text{max}} \) of TNF-\(\alpha\) relative to the control

**TABLE 1**

Dose-independent (25–200 mg/kg) estimated PK parameters of susalimod following i.p. administration of susalimod to female CD-1 mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k ) (min(^{-1}))</td>
<td>0.13</td>
<td>23</td>
</tr>
<tr>
<td>( V_e ) (l/kg)</td>
<td>0.26</td>
<td>6.6</td>
</tr>
<tr>
<td>( V_{\text{max}} ) (( \mu )mol/min·kg)</td>
<td>5.3</td>
<td>25</td>
</tr>
<tr>
<td>( K_m ) (( \mu )M)</td>
<td>70</td>
<td>38</td>
</tr>
<tr>
<td>( CL ) (l/min·kg)</td>
<td>0.0018</td>
<td>17</td>
</tr>
<tr>
<td>( V_i ) (l/kg)</td>
<td>0.37</td>
<td>36</td>
</tr>
<tr>
<td>( k ) (min(^{-1}))</td>
<td>0.027</td>
<td>20</td>
</tr>
</tbody>
</table>

\( V_{\text{max}} \) and \( K_m \) relates to bile clearance; \( CL_{\text{bile}} = \frac{V_{\text{max}}}{K_m + C} \).
Administration of LPS to female CD-1 mice induced high serum levels of the proinflammatory cytokines TNF-\(\alpha\) and IL-6. We found that coadministration of LPS and susalimod lead to a dose-dependent reduction in the TNF-\(\alpha\) exposure, whereas almost no effect on IL-6 was seen. In addition, 200-mg/kg susalimod induced elevated serum levels of the anti-inflammatory cytokine IL-10. These findings are similar to the effect of the chemical analog sulfasalazine on circulating cytokines in RA patients (Danis et al., 1992). During 6 months of sulfasalazine treatment, a clear decrease in circulating cytokines was demonstrated, whereas no effect on circulating IL-6 was seen. Hence, our results indicate that susalimod has a potential use as a pharmacologically active DMARD.

As previously mentioned, LPS administration in the mouse is known to very rapidly induce TNF-\(\alpha\) synthesis in monocytes. This process has been described to occur by the following chain of events: 1) LPS binds to LPS-binding protein; 2) the LPS-binding protein complex interacts with CD14 on the cell surface; 3) following signal transduction, the TNF-\(\alpha\) gene is transcribed to TNF-\(\alpha\) mRNA; 4) mRNA is either degraded or translated into a precursor protein monomer of TNF-\(\alpha\); and 5) precursor TNF-\(\alpha\) is inserted into the cell membrane, modified by membrane enzymes (trimmer formation), and released as mature TNF-\(\alpha\) (Giroir, 1993; Remick, 1995; Su et al., 1995). The successful fit of the proposed PK-PD model to the experimental data indicated an inhibitory effect of susalimod on the synthesis rate of TNF-\(\alpha\), although the present study design did not allow a more specific identification of
which step in the LPS-induced TNF-α synthesis that was affected. Theoretically, either of the following mechanisms is possible: inhibition of the transcription to TNF-α mRNA [as with glucocorticoids (DeForge et al., 1990) and pentoxifylline (Strieter et al., 1988; DeForge et al., 1990)], inhibition of the translation of mRNA to precursor TNF-α [as with glucocorticoids (Han et al., 1990)], or induction of the mRNA degradation [as with thalidomide (Moreira et al., 1993)].

The biological relevance of the proposed PD model was supported by the estimated duration of the TNF-α synthesis (~70 min), which is in good agreement with a previous finding, indicating that the expression of TNF-α mRNA is very rapid but transient (Wollenberg et al., 1993). TNF-α mRNA levels have been reported to peak already at 15 min after LPS challenge and then to decrease to baseline at 1 h. Also, the predicted peak serum levels of TNF-α at ~90 min after LPS administration are in accordance with previous in vivo data in the mouse (Remik et al., 1989).

Apart from describing the concentration-effect relationship and, at least to some extent, having contributed to our understanding of the mechanism of action, this PK-PD model also may be used for prediction of outcomes because the study was designed so that dose-independent parameter estimates could be obtained. The doses used allowed an investigation of the PK of susalimod over a wide concentration range and also covered a large part of the pharmacological effect intensity interval. The nonlinear kinetics behavior of susalimod was modeled by a Michaelis-Menten elimination model. It was estimated that the maximum bile clearance was 76 ml/min·kg. Although previously reported clearance values in the mouse, calculated from AUC following i.v. dosing, are much lower (3.6 ml/min·kg at 50 mg/kg and 7.4 ml/min·kg at 25 mg/kg), clearance data obtained in the dog (1.6 ml/min·kg at 50 mg/kg while 15-fold higher, 20 ml/min·kg at 5 mg/kg) (Påhlman et al., 1998) support the conclusions from the present model that clearance may be very high at low susalimod concentrations (<K_m). However, the accuracy of the estimates of V_{max} and K_m may be questioned because they were found to be sensitive to the assumption of equal rates of absorption for the i.p. administered dose and the enterohepatically reabsorbed drug. A more complex model with the rate of reabsorbed drug modeled as a separate parameter was tested, but was rejected because of the goodness-of-fit criteria stated previously. It should be noted, however, that the more complex PK model generated almost identical PK-PD parameter estimates as the model presented herein (data not shown).

The fact that total concentration of susalimod was used in the PK data analysis and not the unbound concentration may have influenced the results because the possible saturation in binding to plasma albumin was not taken into account. As previously mentioned, susalimod is very highly bound in plasma (>99%) in both animals and humans. In vitro protein-binding studies in mouse plasma have shown that the fraction unbound increased from 0.78% at 25 μM to 0.91% at 250 μM, and one can anticipate a further decrease in binding as the susalimod concentration gets close to and exceeds the plasma albumin levels.

Nevertheless, one can conclude that the proposed PK-PD model accurately described the concentration-effect relationship between the immunomodulating agent susalimod and the experimentally induced elevation of proinflammatory cytokine TNF-α serum levels. The results suggest that susalimod inhibits the rate of TNF-α synthesis with an estimated IC_{50} of 293 μM in the mouse.

Acknowledgments

We thank Lars Engblom (in vivo experiments and cytokine analysis) and Marina Edström (susalimod analysis).

References


Påhlman I, Edholm M, Kankaanranta S and Odell M-L (1998) Pharmacokinetics of susalimod in the mouse, calculated from AUC following i.v. dosing, are in accordance with previous in vivo data in the mouse (Remik et al., 1989).

Påhlman I, Edholm M, Kankaanranta S and Odell M-L (1998) Pharmacokinetics of susalimod in the mouse, calculated from AUC following i.v. dosing, are in accordance with previous in vivo data in the mouse (Remik et al., 1989).

Peter Gozzi, Pharmacia & Upjohn AB, SE-11287 Stockholm, Sweden. E-mail: peter.gozzi@eu.pnu.com

Send reprint requests to: Peter Gozzi, Pharmacia & Upjohn AB, SE-11287 Stockholm, Sweden. E-mail: peter.gozzi@eu.pnu.com

203

PK-PD Modeling of Susalimod and TNF-α

Nevertheless, one can conclude that the proposed PK-PD model accurately described the concentration-effect relationship between the immunomodulating agent susalimod and the experimentally induced elevation of proinflammatory cytokine TNF-α serum levels. The results suggest that susalimod inhibits the rate of TNF-α synthesis with an estimated IC_{50} of 293 μM in the mouse.