Stereospecific Determination, Chiral Inversion In Vitro and Pharmacokinetics in Humans of the Enantiomers of Thalidomide

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ABSTRACT
The purposes of this work were (1) to develop a high performance liquid chromatographic (HPLC) assay for the enantiomers of thalidomide in blood, (2) to study their inversion and degradation in human blood, and (3) to study the pharmacokinetics of (+)-(R)- and (−)-(S)-thalidomide after oral administration of the separate enantiomers or of the racemate to healthy male volunteers. The enantiomers of thalidomide were determined by direct resolution on a tribenzoyl cellulose column. Mean rate constants of chiral inversion of (+)-(R)-thalidomide and (−)-(S)-thalidomide in blood at 37°C were 0.30 and 0.31 h⁻¹, respectively. Rate constants of degradation were 6.17 and 0.18 h⁻¹. There was rapid interconversion in vivo in humans, the (+)-(R)-enantiomer predominating at equilibrium. The pharmacokinetics of (+)-(R)- and (−)-(S)-thalidomide could be characterized by means of two one-compartment models connected by rate constants for chiral inversion. Mean rate constants for in vivo inversion were 0.17 h⁻¹ (R to S) and 0.12 h⁻¹ (S to R) and for elimination 0.079 h⁻¹ (R) and 0.24 h⁻¹ (S), i.e., a considerably faster rate of elimination of the (−)-(S)-enantiomer. Putative differences in therapeutic or adverse effects between (+)-(R)- and (−)-(S)-thalidomide would to a large extent be abolished by rapid interconversion in vivo.

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KEY WORDS: thalidomide enantiomers, stereospecific analysis, high-performance liquid chromatography, in vitro kinetics, chiral inversion, stereoselective pharmacokinetics

Thalidomide (α-phthalimidoglutarimide) (Fig. 1) has a chiral center and exists as a racemate of the (+)-(R)- and (−)-(S)-enantiomers. The racemate was introduced as a sleep-inducing agent in 1956, but it was withdrawn in 1961 for causing fetal malformations. ¹,² A few years later, thalidomide was reported to be remarkably active against severe immunological manifestations in some types of leprosy.³ Thalidomide has proved to be an efficacious antiinflammatory agent in a variety of skin and mucous membrane disorders.²,⁴ Its immunomodulating actions are also utilized in the prevention and treatment of graft-vs.-host disease after bone marrow transplantation.⁵,⁶ The therapeutic dose of rac-thalidomide is normally 100–400 mg/day, which may cause severe drowsiness.¹⁻⁴ In graft-vs.-host disease, doses up to 1600 mg/day may be used.⁵,⁶ Known or possible pregnancy is, of course, an absolute contraindication to the use of thalidomide. Currently, however, poorly supervised use of thalidomide against leprosy is causing an unknown and possibly large number of birth defects in Brazil.⁶,⁷

In the modern use of thalidomide, its sedative/hypnotic action must be regarded as an unwanted side effect. Other adverse effects of thalidomide include potentially severe peripheral neuropathy during regular use,²,⁴,⁹ as well as constipation, xerostomia, and skin rash.²

It is presently unclear whether any of the actions of racemic thalidomide can be separated out using a pure enantiomer.² Findings in the literature are contradictory. Separately synthesized thalidomide enantiomers given orally produced similar hypnotic effects in mice and similar teratogenic effects in rabbits.¹⁰ In contrast, when chromatographically separated enantiomers (of possibly higher optical purity) were given intraperitoneally to mice and rats, (−)-(S)-thalidomide caused dose-dependent teratogenicity in both species, while (+)-(R)-thalidomide was devoid of effect.¹¹ Regarding the other important adverse effect of thalidomide, i.e., peripheral neuropathy, nothing is known about the relative activities of the enantiomers. In animal models of graft-vs.-host disease, (−)-(S)-thalidomide was superior to (+)-(R)-thalidomide in preventing splenomegaly in chicken embryos,¹² but in rats no difference between the enantiomers was seen.¹³

Attempts to separate the various pharmacological effects of drugs such as thalidomide using pure enantiomers become rather meaningless if a singly administered enantiomer is rapidly racemized in vivo. Interconversion of enantiomers under
physiological conditions has been observed, e.g., with oxazepam, amfepramone and congeners, and with some analogs of thalidomide, and, as demonstrated with the 2-arylpropionic acid antiinflammatory agents, unidirectional chiral inversion may occur in vivo. Conversely, if the disposition of the drug is markedly stereoselective so that one enantiomer will become the predominant species in vivo after administration of the racemate, the body may also be exposed chiefly to the desired (or the undesired) enantiomer.

Dosing regimens of thalidomide have been based on early clinical studies and case reports rather than on its pharmacokinetic behavior. One pharmacokinetic study has been reported, in which eight healthy male volunteers were given a single oral dose of 200 mg of the racemate. From plasma data, the mean C$_{\text{max}}$ was estimated at 1.15 $\mu$g/ml, t$_{\text{max}}$ at 4.39 h, and mean elimination half-life (in a one-compartment model) at 8.70 h. Renal excretion was 0.6% of dose. Thalidomide is mainly eliminated by spontaneous hydrolysis in blood and tissues, but metabolism by aromatic hydroxylation has also been found.

The pharmacokinetics of the enantiomers of thalidomide have, however, not been described. In view of their rapid interconversion in vitro in biological media, it is likely that chiral inversion would be observed also in vivo. The paucity of pharmacokinetic data is presumably due to the lack of a rapid stereospecific assay for thalidomide in biological materials. Liquid chromatographic methods suitable for preparative separation of thalidomide enantiomers have been described, but only very recently has a high performance liquid chromatographic (HPLC) assay applicable to plasma samples been reported.

The purposes of this work were, therefore, (1) to develop a rapid and sensitive HPLC assay for the enantiomers of thalidomide in blood, (2) to study the inversion and degradation of the enantiomers in vitro (in human blood), and (3) to study the pharmacokinetics of (+)-(R)- and (−)-(S)-thalidomide after oral administration of the separate enantiomers or of the racemate to healthy volunteers.

**MATERIALS AND METHODS**

**Stereospecific HPLC Assay**

Racemate and enantiomers of thalidomide were kindly supplied by Grünenthal GmbH (Stolberg/Rheinland, Germany) and phenacetin was of European Pharmacopoeia quality. All stock solutions were prepared in methanol and kept at $-25^\circ$C. Diethyl ether, dichloromethane, and n-hexane were purchased from E. Merck (Darmstadt, Germany). Methanol and 99.5% ethanol were of Pharmacoepia quality. Water was freshly distilled and collected in a stainless steel vessel. Liquid chromatographic systems consisted of LDC/Milton Roy (Rivera Beach, FL) Constametric III pumps, Rhodyne 7725 loop injectors with 20 $\mu$l loops, and Spectrmonitor III variable-wavelength UV detectors. For the stereospecific assay, a tribenzoyl cellulose column (Chiral Tribencel, Macherey-Nagel, Düren, Germany; 250 x 4 mm I.D.), protected by a LiChrospher C-18 guard column (E. Merck; 4 x 4 mm), was used, and the mobile phase was methanol with 10%-25% of 99.5% ethanol. Flow rate was 0.5 ml/min and detection wavelength was 220 nm.

Immediately after collection, all blood samples were stabilized by the addition of an equal volume of Sörensen's citrate buffer, 0.025 M, pH 1.5. After addition of 50 $\mu$l of internal standard solution (phenacetin, 100 $\mu$g/ml), samples (1.0-4.0 ml of blood-buffer mixture) were extracted with 5.0 ml of either diethyl ether or dichloromethane:n-hexane (1:1) as previously described for rac-thalidomide. The organic layer was transferred to another tube, the solvent was evaporated under a stream of dry air, the residue was taken up in 100 $\mu$l of methanol, and 20 $\mu$l of this solution was injected into the chromatograph for determination of the thalidomide enantiomer ratio. The remainder of the extract was then used for determination of the total concentration of thalidomide by nonstereospecific HPLC as previously described. Due to the risk of interference in the chromatograms, possible contaminants, e.g., plasticware, detergents, and community water, were avoided. Glass-stoppered tubes washed with distilled water and 99.9% ethanol were used throughout.

The extraction yields of thalidomide and phenacetin from blood-buffer with dichloromethane:n-hexane (1:1) were determined as previously described using samples spiked with 1 $\mu$g/ml of rac-thalidomide. Extraction yields using diethyl ether have been reported previously. Standard curves were prepared from 1 ml of blood-buffer solution (1:1), spiked with internal standard (5 $\mu$g) and thalidomide to a total amount of 1.0 $\mu$g with R/S or S/R ratios of 0, 0.063, 0.125, 0.33, 0.67, and 1.0. Within-day accuracy and precision in blood-buffer samples was checked by assay of series of eight samples (1 ml) spiked with various concentrations and ratios of thalidomide enantiomers (see Table 1).

During the actual assays in the kinetic studies, one-point standardization against worked-up samples of rac-thalidomide was used. The between-day coefficient of variation (C.V.) was calculated from the S/R peak height ratios from these samples. In addition, the between-day C.V. of the nonstereospecific assay was monitored using a pool spiked with 0.40 $\mu$g/ml of rac-thalidomide.

Possible racemization of (−)-(S)-thalidomide in methanol stock solutions (100 $\mu$g/ml) protected from light and kept in the freezer ($-25^\circ$C) was monitored at 1, 2, 3, 6, 8, 12, 16, 27, 48, and 221 days by direct injection into the chromatograph. Stereorechemical stability of (+)-(R)- and (−)-(S)-thalidomide was also investigated by spiking freshly drawn heparinized
human blood to a total concentration of 1.0 μg/ml. An equal volume of citrate buffer solution was added, and the samples were kept at -25°C in the dark. Four samples were analyzed at once and another four after 100 days.

In Vitro Inversion and Degradation of (+)-(R)- and (-)-(S)-Thalidomide in Blood at 37°C

(+)-(R)- or (-)-(S)-thalidomide was added to freshly drawn, heparinized human blood from four healthy volunteers to a concentration of 5 μg/ml. Mixtures were dispensed in glass tubes filled with CO₂-O₂ (95:5) (for control of pH), sealed, and incubated at 37°C in a water bath. Duplicate samples were taken at 0.083, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 h. Simultaneously, incubations were done with rac-thalidomide and sampling at 1, 4, 7, 9, and 12 h. An equal volume of citrate buffer solution was added to stop the reactions, and samples were stored at -25°C until analyzed (within 10 days). The pH of the spiked blood was checked at 0, 1, 4, 7, 9, and 12 h, using an ABL 3300 automatic blood gas laboratory (Radiometer, Copenhagen, Denmark).

Human Pharmacokinetics—Trial Protocol

The study was approved by the Ethics Committee of the University of Lund and by the Swedish Medical Products Agency. Six healthy male volunteers (aged 25-40 years, weight 71-100 kg), who were free of medication and had no history of allergy to drugs, gave informed written consent to the study. They received oral thalidomide as pure (+)-(R)- or (-)-(S)-enantiomer or as racemate on three occasions separated by at least 1 week, in a randomized 3 x 3 Latin square design. The dose was 1.0 mg/kg of either (+)-(R)- or (-)-(S)-thalidomide or 1.5 mg/kg of the racemate. Hard gelatin capsules containing the individual doses of drug and lactose q.s. were prepared by the laboratory in charge of randomization (S.B.), while subjects and investigators participating in the study sessions were blind to treatment. Subjects were fasting since the evening before (for at least 10 h). At approximately 8:30 A.M., they swallowed the capsule with 200 ml of water and remained supine during the rest of the day (for 8-10 h), except that they were allowed up to take a light lunch after 4 h. Sedation and other possible drug effects were noted concomitantly with blood sampling. Samples were taken from an antecubital vein into heparinized tubes before and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, 23, and 24 h after drug intake. Each blood sample was directly mixed with an equal volume of citrate buffer and frozen. Blood-buffer mixtures were stored at -25°C until analysis, for a maximum of 10 days.

Pharmacokinetic Analysis

Rate constants for in vitro inversion and degradation shown in Figure 1 were estimated by the fitting of a two-compartment pharmacokinetic model with elimination from both compartments to the measured blood concentrations of (+)-(R)- and (-)-(S)-thalidomide during incubation of either enantiomer. Model equations were coded in the SAS software (SAS Institute, Cary, NC), and the model was fitted simultaneously to the four concentration curves obtained in each experiment, with the reciprocals of the predicted concentrations as weighting factors.

From the in vivo data, apparent terminal half-lives of the enantiomers were estimated by nonlinear regression using the RSTRIP software (MicroMath, Salt Lake City, UT). Area under the curve (AUC) was calculated by the logarithmic trapezoidal method from 0 to infinity using the MKMODEL software (N. Holford, Auckland, New Zealand). A pseudoequilibrium R/S blood concentration ratio was calculated for each subject as an average over the 10–24 h concentration data after administration of the racemate.

The pharmacokinetic model shown in Figure 2 was used to estimate the rate constants for absorption, inversion, and elimination of the two enantiomers. Compartment volumes, Vₐ,R and Vₛ, could be estimated only as apparent values, i.e., volume divided by effective bioavailability (F), since blood concentration data after intravenous administration could not be obtained. The model was coded in the SAS software, and estimations were made by the NLIN procedure. First, the concentrations of (+)-(R)- or (-)-(S)-thalidomide obtained in the two sessions where each enantiomer was administered separately were used to estimate all parameters of the model, fitting the four concentration curves simultaneously. For each subject, iteratively reweighted least squares estimations were performed using first the reciprocals of the predicted concentrations and then of the squares of the predicted con-
concentrations as weighting factors. The choice between these two weighting factors for each subject was based on the $r^2$ value of the fit and on inspection of the residuals.

In a second step, the rate of absorption and the apparent volumes of distribution of the enantiomers after administration of rac-thalidomide were estimated for each subject using the previously found disposition parameters (and weighting factors) as fixed values.

Statistics

The comparisons of estimated pharmacokinetic parameters were tested by the Shapiro-Wilk statistics, setting $P < 0.10$ as the limit for rejection of the null hypothesis of normal distribution. Normally distributed parameters were compared by one-way analysis of variance (ANOVA) followed by Dunnett's multiple range test. The accuracy and precision of the assay are shown in Table 1. The between-day C.V. of the stereospecific assay was 1.6% (n = 31) and that of the assay for total thalidomide was 8.2% (n = 16).

The (+)-(R)- and (-)-(S)-thalidomide supplied proved to contain less than 0.7% of the other enantiomer. There was less than 1% loss of optical purity in the stock solutions of pure enantiomer kept at $-25^\circ$C after 221 days. There was no detectable racemization of thalidomide enantiomers in blood-buffer solutions stored for 100 days at $-25^\circ$C.

RESULTS

Stereospecific HPLC Assay

Representative chromatograms are given in Figure 3. The capacity factors ($k'$) of (+)-(R)- and (-)-(S)-thalidomide were 2.4 and 3.1, respectively, when the mobile phase consisted of 100% methanol. They rose to 2.6 and 3.2 with the admixture of 10% ethanol and to 2.8 and 3.6 with 25% ethanol in the mobile phase. In the last case, a new sample could be injected every 15 min. The columns proved quite stable and the chromatography very reproducible. In fact, a single analytical column was used for the entire work described here.

The extraction yield of rac-thalidomide from acidified blood, using dichloromethane:n-hexane (1:1), was 96 ± 2.0% (mean ± S.D.). The corresponding value for phenacetin was 91 ± 7.0%. This extraction medium was normally used. The standard curves prepared from thalidomide enantiomers in the ratios of 0.063 to 1.0 were linear (generally r = 0.999).

In Vitro Inversion and Degradation of (+)-(R)- and (-)-(S)-Thalidomide in Blood at 37°C

The inversion and degradation of the (+)-(R)- and (-)-(S)-thalidomide enantiomers in vitro is illustrated in Figure 4. Estimated rate constants are given in Table 2. Incubation of rac-thalidomide in blood caused a slight shift in equilibrium between the enantiomers. Steady-state R/S concentration ratios, calculated as the mean of the 9 and 12 h values, are also shown in Table 2.

Human Pharmacokinetics

During the first 8–10 h of the study sessions, the subjects became mildly to markedly sedated or fell asleep. No other
TABLE 2. In vitro rate constants and corresponding half-lives of inversion and degradation of enantiomers of thalidomide in human blood, and equilibrium R/S concentration ratios (obtained in separate incubations of rac-thalidomide)∗

<table>
<thead>
<tr>
<th>Subject</th>
<th>Rate of inversion (h⁻¹)</th>
<th>Rate of degradation (h⁻¹)</th>
<th>R/S equilib. ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k_RS</td>
<td>k_SR</td>
<td>k_RS</td>
</tr>
<tr>
<td>A</td>
<td>0.31</td>
<td>0.31</td>
<td>0.14</td>
</tr>
<tr>
<td>B</td>
<td>0.32</td>
<td>0.34</td>
<td>0.22</td>
</tr>
<tr>
<td>C</td>
<td>0.28</td>
<td>0.29</td>
<td>0.16</td>
</tr>
<tr>
<td>D</td>
<td>0.29</td>
<td>0.30</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean</td>
<td>0.30</td>
<td>0.31</td>
<td>0.17</td>
</tr>
<tr>
<td>SD</td>
<td>0.017</td>
<td>0.019</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Corresponding half-lives (h)

| Mean | 2.31 | 2.25 | 4.33 | 3.82 | 1.07         |
| S.D. | 0.13 | 0.13 | 0.84 | 0.57 | 0.05         |

∗Abbreviations as in Figure 1. Mean values with different letters are significantly different (P < 0.05) by ANOVA and Duncan’s multiple range test. The results of the in vitro incubations in blood confirm previous findings that thalidomide is rapidly hydrolyzed16,17,22 and that the enantiomers rapidly interconvert19 in biological media at physiological pH. A modest stereoselective influence of blood constituents was apparent as a slightly higher rate of degradation of the (-)-(S)- than of the (+)-(R)-enantiomer, with a consequent shift in pseudoequilibrium concentration ratio. There was also a clear difference between rates of inversion and of degradation, inversion being the faster process for both enantiomers.

The study of the in vivo pharmacokinetics of thalidomide was hampered by the lack of an intravenous formulation. Attempts to adequately improve the poor solubility and stability of thalidomide in water have met with only limited success.13 Our chief aims were to describe the disposition of the enantiomers of thalidomide, i.e., determine their rate constants for inversion and elimination, and to investigate to what extent administration of a single enantiomer can reduce the actual exposure of the subject to the other enantiomer. These aims could be realized even though most conventional pharmacokinetic parameters could not be estimated.
Kinetics of the Enantiomers of Thalidomide

Subject 4
(+)-(R)-Thalidomide

Subject 4
(-)-(S)-Thalidomide

Subject 6
(+)-(R)-Thalidomide

Subject 6
(-)-(S)-Thalidomide

Fig. 5. Blood concentration curves of (+)-(R)-thalidomide (filled symbols) and (-)-(S)-thalidomide (open symbols) after oral administration of the indicated enantiomer (both 1.0 mg/kg) to two subjects. The best model-fit ($r^2 = 0.984$, subject 4) and the worst ($r^2 = 0.935$, subject 6) are shown. In both cases, the chosen weighting factor was 1/(predicted concentration)^2.

Discussion:

Concentration curves from the former experiments were used for the estimation of the rate constants for inversion and elimination, since the shorter absorption phases would have less impact on these estimations.

The slower absorption of (+)-(R)- and (-)-(S)-thalidomide when given as the racemate was confirmed by model-fitting. It is probably due to a slower dissolution of racemic thalidomide than of the enantiomers, which in turn would depend on the considerably lower aqueous solubility of the racemate, 50 mg/l, than of the separate enantiomers, 250 mg/l.13,23 The $k_a$ values of 0.33 and 0.38 h$^{-1}$ that we found for absorption of (+)-(R)- and (-)-(S)-thalidomide from capsules containing the racemate correspond to a mean absorption half-life of 2.0 h. A similar mean half-life of 1.7 h has been reported for the absorption of thalidomide from tablets.15

In the earlier pharmacokinetic study of thalidomide,15 in which no distinction was made between the enantiomers, apparent clearance and volume of distribution were estimated assuming 100% bioavailability. In describing the pharmacokinetics of the enantiomers, this assumption is untenable. When reversible biotransformation occurs, even if concentration data after intravenous administration are used, the true clearance of drug cannot be calculated by the standard formula of dose/AUC.24 This is because "dose" is not simply the administered amount of drug but also incorporates an amount formed in vivo, in the present case by inversion of the opposite enantiomer. Consequently, with oral administration bioavailability also becomes a complex parameter. Each enantiomer is bioavailable both as such and as an amount formed by inversion of the other enantiomer. This is illustrated in our pharmacokinetic model (Fig. 2). The input of drug into, e.g., $V_{d,R}$ is influenced by $k_{a,R}$ and $k_{i,S}$ (and indirectly by $k_{R}$) if (+)-(R)-thalidomide is administered; by $k_{a,S}$ and $k_{i,R}$ (and $k_{R}$) if (-)-(S)-thalidomide is given; and by $k_{a,R}$, $k_{a,S}$, $k_{i,S}$ (and $k_{i,R}$) if the racemate is administered. In the last case, the bioavailability of the enantiomer predominating at steady state could easily exceed 100%. Interpretation of the estimated compartmental volumes ($V_{d}/F$) is therefore ambiguous. The difference between $V_{d,R}/F$ and $V_{d,S}/F$ may be due to an actual difference in distribution between the enantiomers, but the lower value of $V_{d,R}/F$ may also reflect a higher effective bioavailability of the kinetically favored (+)-(R)-enantiomer.

A theoretically more attractive pharmacokinetic model would include rate constants for presystemic inversion and elimination and possibly a peripheral (distribution) compartment for each enantiomer. Rate constants for presystemic processes were initially included in the model but were found to be highly correlated and had to be rejected. This is hardly
TABLE 3. In vivo disposition (inversion and elimination) rate constants of (+)-(R)- and (-)-(S)-thalidomide, with 95% confidence intervals

<table>
<thead>
<tr>
<th>Subject</th>
<th>Rate of inversion $k_{IR}$ (h$^{-1}$)</th>
<th>Rate of elimination $k_{IE}$ (h$^{-1}$)</th>
<th>Rate of inversion $k_{IS}$ (h$^{-1}$)</th>
<th>Rate of elimination $k_{ES}$ (h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.18 (0.12-0.25)</td>
<td>0.08 (0.03-0.12)</td>
<td>0.000$^a$ (-0.05-0.05)</td>
<td>0.32 (0.14-0.51)</td>
</tr>
<tr>
<td>2</td>
<td>0.15 (0.10-0.21)</td>
<td>0.13 (0.09-0.17)</td>
<td>0.095 (0.06-0.13)</td>
<td>0.22 (0.18-0.26)</td>
</tr>
<tr>
<td>3</td>
<td>0.18 (0.12-0.24)</td>
<td>0.18 (0.12-0.23)</td>
<td>0.125 (0.09-0.16)</td>
<td>0.20 (0.15-0.25)</td>
</tr>
<tr>
<td>4</td>
<td>0.17 (0.14-0.19)</td>
<td>0.11 (0.09-0.12)</td>
<td>0.092 (0.08-0.11)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>5</td>
<td>0.16 (0.13-0.18)</td>
<td>0.13 (0.10-0.16)</td>
<td>0.069 (0.05-0.09)</td>
<td>0.27 (0.21-0.34)</td>
</tr>
<tr>
<td>6</td>
<td>0.19 (0.12-0.26)</td>
<td>0.12 (0.08-0.16)</td>
<td>0.095 (0.03-0.16)</td>
<td>0.24 (0.19-0.29)</td>
</tr>
<tr>
<td>Mean</td>
<td>0.17</td>
<td>0.12</td>
<td>0.079</td>
<td>0.24</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.02</td>
<td>0.03</td>
<td>0.043</td>
<td>0.05</td>
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Corresponding half-lives (h)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Rate of inversion $k_{IR}$ (h$^{-1}$)</th>
<th>Rate of elimination $k_{IE}$ (h$^{-1}$)</th>
<th>Rate of inversion $k_{IS}$ (h$^{-1}$)</th>
<th>Rate of elimination $k_{ES}$ (h$^{-1}$)</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>4.07</td>
<td>5.89</td>
<td>7.54</td>
<td>2.91</td>
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<tr>
<td>B</td>
<td>1.64</td>
<td>1.58</td>
<td>1.58</td>
<td>0.49</td>
</tr>
</tbody>
</table>

"Abbreviations as in Figure 2. Mean values with different letters are significantly different ($P < 0.05$) by ANOVA and Duncan's multiple range test.

The fit converged on the lower limit (0.0001) of this parameter. Not included in the calculation of mean half-lives.

Fig. 6. Blood concentration curves of (+)-(R)-thalidomide (filled symbols) and (-)-(S)-thalidomide (open symbols) after oral administration of racemic thalidomide, 1.5 mg/kg, to two subjects. Model-fitted curves are based on the rate constants for inversion and elimination determined after administration of the pure enantiomers to these subjects. Only absorption rate constants, possible lag times, and compartmental volumes of distribution were estimated. Subject 1 illustrates absorption without time lag, while for subject 5 a lag time of 42 min was estimated.

surprising since we have no measured data for presystemic processes. As for peripheral compartments, there were no discernible distribution phases in the curves, these being masked by the relatively slow absorption and the "distribution" in the shape of reversible inversion. Also, from a statistical point of view, the considerably increased number of parameters would most probably outweigh any improvement in the fits, especially since a plausible model would have to include elimination from and inversion between the peripheral compartments.

Inversion of the enantiomers was faster in vitro than in vivo (Tables 2 and 3). In this context it should be noted that the estimated compartment volumes ($V_i/F$) considerably exceed the physiological blood volume (0.07 l/kg) and that with any plausible value for $F$ (i.e., above approximately 0.1) the actual volumes of distribution of the enantiomers must also do so. Some thalidomide is consequently distributed extravascularly to sites where inversion appears to be slower than in blood. When elimination in vivo is compared to degradation (presumably only by hydrolysis) in vitro, the rate was slower in vivo than in vitro for the (+)-(R)-enantiomer, while (-)-(S)-thalidomide was eliminated faster in vivo than it was hydrolyzed in vitro. Since the rates of hydrolysis of (+)-(R)- and (-)-(S)-thalidomide in blood were only marginally different, it is unlikely that the threefold difference in rate of elimination of the enantiomers in vivo would be due only to stereoselective facilitation (or inhibition) of nonenzymic hydrolysis. The data suggest a greater extent of metabolism for the (-)-(S)-enantio-mer.

The mean apparent terminal half-life of the thalidomide enantiomers was 4.7 h (when equilibrium between the enantiomers has been established, their half-lives will be identical). Mean elimination half-life of thalidomide has previously been reported as 8.7 h, with a considerable interindividual variability: 3.0–14.6 h. Even after intravenous administration of a drug, the estimation of terminal half-life is markedly dependent on the blood sampling schedule. The study protocol used in the cited report entailed frequent blood sampling over 12 h followed by a single sample at 24 h. With maximal plasma concentrations at 2–6 h, the estimates of elimination half-lives may have been based on rather sparse data. Additional blood sampling at 16, 20, and 24 h and calculation of
TABLE 4. Apparent volumes of distribution (Vd/F) and absorption rate constants of (+)-(R)- and (-)-(S)-thalidomide estimated after administration of separate enantiomers (E) or of racemate (rac)\(^a\)

<table>
<thead>
<tr>
<th>Subject</th>
<th>(V_{d,R}/F)</th>
<th>(V_{d,S}/F)</th>
<th>(k_{a,R})</th>
<th>(k_{a,S})</th>
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<tbody>
<tr>
<td>1</td>
<td>0.71</td>
<td>1.17</td>
<td>1.25</td>
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<td>0.74</td>
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<td>0.58</td>
<td>0.71</td>
<td>0.34</td>
<td>0.17</td>
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<td>0.46</td>
<td>0.79</td>
<td>2.43</td>
<td>0.22</td>
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<tr>
<td>5</td>
<td>0.75</td>
<td>0.99</td>
<td>2.29</td>
<td>0.33</td>
</tr>
<tr>
<td>6</td>
<td>0.55</td>
<td>0.93</td>
<td>0.27</td>
<td>0.33</td>
</tr>
<tr>
<td>Mean</td>
<td>0.60</td>
<td>0.89</td>
<td>0.79(^b)</td>
<td>0.33</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.11</td>
<td>0.17</td>
<td>0.16</td>
<td>0.17</td>
</tr>
</tbody>
</table>

\(^a\)Abbreviations as in Figure 2. Mean values with different letters are significantly different (\(P < 0.05\)) by ANOVA and Duncan’s multiple range test. After administration of the racemate, but not after the enantiomers, lag times for absorption were observed: 0.38 h in subject 2, 0.32 h in subject 3, and 0.70 h in subject 5.

\(^b\)\(k_a\) values after administration of the enantiomers were not normally distributed, thus median values are given.

TABLE 5. AUC (\(\mu g \cdot h/ml\)) values, calculated by the trapezoidal method, for (+)-(R)- and (-)-(S)-thalidomide after administration of the separate enantiomers or of racemic thalidomide in six subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>(AUC_{R})</th>
<th>(AUC_{S})</th>
<th>(AUC_{R})</th>
<th>(AUC_{S})</th>
<th>(AUC_{R})</th>
<th>(AUC_{S})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.47</td>
<td>3.65</td>
<td>1.59</td>
<td>1.75</td>
<td>14.2</td>
<td>9.66</td>
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<tr>
<td>2</td>
<td>9.08</td>
<td>3.01</td>
<td>3.51</td>
<td>4.63</td>
<td>10.8</td>
<td>6.44</td>
</tr>
<tr>
<td>3</td>
<td>7.60</td>
<td>2.94</td>
<td>3.69</td>
<td>5.01</td>
<td>11.5</td>
<td>7.95</td>
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<td>4</td>
<td>10.13</td>
<td>3.30</td>
<td>3.40</td>
<td>5.09</td>
<td>12.0</td>
<td>7.73</td>
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<tr>
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<td>7.32</td>
<td>2.15</td>
<td>2.44</td>
<td>3.20</td>
<td>10.5</td>
<td>6.03</td>
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<tr>
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<td>7.92</td>
<td>2.60</td>
<td>2.77</td>
<td>3.75</td>
<td>11.3</td>
<td>6.73</td>
</tr>
<tr>
<td>Mean</td>
<td>8.59</td>
<td>2.94</td>
<td>2.90</td>
<td>3.90</td>
<td>11.7</td>
<td>7.42</td>
</tr>
<tr>
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<td>0.52</td>
<td>0.80</td>
<td>1.29</td>
<td>1.34</td>
<td>1.32</td>
</tr>
</tbody>
</table>

\(^a\)For easier comparison, AUC values after rac-thalidomide have been recalculated for a total dose of 2.0 mg/kg.

CONCLUSION

From a practical point of view, the most important conclusion of this study is that putative differences in therapeutic or adverse effects between (+)-(R)- and (-)-(S)-thalidomide would to a large extent be abolished by rapid interconversion in vivo of separately administered enantiomers.

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