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Intravenous Formulations of the Enantiomers of Thalidomide: Pharmacokinetic and Initial Pharmacodynamic Characterization in Man

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Abstract

Thalidomide, a racemate, is coming into clinical use as an immunomodulating and anti-inflammatory drug. These effects may chiefly be exerted by *S*-thalidomide, but the enantiomers are interconverted in-vivo. Thalidomide is given orally, although parenteral administration would be desirable in some clinical situations. The aim of this study was to prepare solutions of the enantiomers of thalidomide for intravenous administration and to investigate their pharmacokinetics and sedative effects following infusion in man.

Solubility and stability of the enantiomers in 5% glucose solution was investigated. After a dose-determination experiment in one subject, six healthy male volunteers received *R*- and *S*-thalidomide separately by 1-h infusions in a randomized double-blind cross-over study. Blood was sampled over 22 h and sedative effects were recorded. Blood concentrations of the enantiomers were determined by stereospecific HPLC. A four-compartment model consisting of a two-compartment model for each enantiomer, with elimination from both compartments, connected by rate constants for chiral inversion was fitted to the concentration data, while the sedative effects were correlated with the blood concentrations of *R*- and *S*-thalidomide by means of logistic regression. The enantiomers of thalidomide were chemically stable in solution for at least a week at room temperature. The infusions were well tolerated. Sedation, which was the only observed effect, was related to the blood concentration of *R*-thalidomide. Inter-individual variation in the disposition of the enantiomers was modest (e.g. terminal half-lives ranged between 3·9 and 5·3 h). Pharmacokinetic modelling predicted that varying the infusion time of a fixed dose of *S*-thalidomide between 10 min and 6 h would have little influence on the maximal blood concentration of formed *R*-thalidomide.

To our knowledge this is the first time that thalidomide has been administered intravenously.

Thalidomide was used as a hypnotic/sedative drug for a few years in the late 1950s and early 1960s but it was withdrawn due to teratogenicity and toxic neuropathy (Koch 1984). Because of its immunomodulating effects, which at least in part are due to inhibition of the formation of tumour necrosis factor-α (TNF-α), there is a renewed interest in thalidomide for the treatment of diseases and syndromes with an immunological component (Schuler & Ehninger 1995; Zwingenberger & Wnendt 1996). In 1998 the United States Food and Drug Administration approved thalidomide for the treatment of the cutaneous manifestations of erythema nodosum leprosum, a form of leprosy (Nightingale 1998). Therapeutic effects of thalidomide have also been shown more or less conclusively in a number of other conditions, such as graft-versus-host disease (GVHD; Vogelsang et al 1992), oral or oesophageal aphthous ulcers in patients with HIV infection (Jacobson et al 1997, 1999), Beçhet's disease (Hamuryudan et al 1998), cutaneous lesions of systemic lupus erythematosis (Atra & Sato 1993) and, recently, in multiple myeloma (Singhal et al 1999). Approval for further indications may follow in the rest of the world, as well as in the USA.

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Thalidomide is clinically used as a racemate. The sedative effects are due to the *R*-enantiomer, as demonstrated by logistic regression in human volunteers (Höglund et al 1998). Results of in-vitro experiments suggest that the immunological effects of thalidomide may, on the other hand, chiefly be exerted by the *S*-enantiomer (Nishimura et al 1994; Wnendt et al 1996). Clinically, however, the effects of *R*- and *S*-thalidomide cannot be clearly separated, since the enantiomers undergo rapid chiral inversion in-vivo (Eriksson et al 1995).

Thalidomide is given orally. However, the need for a parenteral formulation has been pointed out in various contexts. Patients with acute GVHD may be unable to take oral medication or may show impaired intestinal absorption of thalidomide (Heney et al 1991; Krenn et al 1992; Boughton et al 1995). In severe cases of oropharyngeal or oesophageal ulceration most patients experience discomfort or pain while eating (Jacobson et al 1999) and taking an oral medication may be difficult. A possible use of thalidomide in the treatment of sepsis also indicates a need for parenteral administration (Schmidt et al 1996).

The poor solubility ($50\,\mu g\,mL^{-1}$) of racemic thalidomide in water is the main obstacle to the preparation of an injectable solution. In addition, spontaneous hydrolysis (Schumacher et al 1965; Eriksson et al 1992, 1998; Eriksson & Björkman 1997; Reist et al 1998) and chiral inversion of the enantiomers (Knoche & Blaschke 1994; Eriksson & Björkman 1997; Eriksson et al 1998; Reist et al 1998) occur in aqueous media with pH values above 4–5. Solubility and stability can be improved by complexation of thalidomide with hydroxypropyl-β-cyclodextrin (Krenn et al 1992) but preparation of injectable solutions by this technique has not been demonstrated.

The aim of this study was to prepare intravenous formulations of *S*- and *R*-thalidomide and to characterize the pharmacokinetics and sedative effects of the enantiomers in man when either of them is given by short-term infusion. A further aim was then to use the obtained pharmacokinetic model to predict the disposition of the enantiomers after different rates of infusion of *S*-thalidomide.

Materials and Methods

HPLC assay

The blood concentrations of the enantiomers of thalidomide were determined by HPLC as described previously (Eriksson et al 1992, 1995; Eriksson & Björkman 1997). The between-day coefficient of variation (CV) for determination of total concentrations (sum of enantiomers) was 5·8% at $0{\cdot}4\,\mu g\,mL^{-1}$ ($n = 10$).

Preparation of solutions for infusion

To determine their solubility, powdered *R*-, *S*- or racemic thalidomide, 5 mg, was added to freshly distilled water or 5% glucose solution, 5 or 10 mL. The suspensions were vortex-mixed for up to 30 min and duplicate samples of the supernatants were taken in sequence. These were filtered through a 0·22-μm syringe filter (Alltech, Deerfield, IL), diluted 1 : 50 with mobile phase and directly injected into the chromatograph for determination of the total concentration of thalidomide. The experiments were performed at room temperature (23°C).

Solutions for infusion of *R*- or *S*-thalidomide, $0{\cdot}20\,mg\,mL^{-1}$, were then prepared from substances (provided by Grünenthal GmbH, Stolberg, Germany) that passed the European Pharmacopoeia test for pyrogenicity and had been shown to contain < 40 microorganisms g^{-1}. Of either enantiomer, 70 mg was added to 350 mL of 5% glucose solution for infusion (pH 4–5) in a glass infusion bottle (Pharmacia & Upjohn, Stockholm, Sweden). The mixture was vigorously shaken and ultrasonicated for approximately 15 min. This procedure was repeated until dissolution was complete (typically four times). The temperature of the water in the ultrasonic bath was allowed to reach a maximum of 33°C. The solution was filtered into a sterile glass infusion bottle under aseptic conditions using a 0·22-μm Millex GS sterile filter (Millipore S.A., Mollsheim, France) to give the final infusion solution. The bottles were labelled with subject and session numbers by an author not taking part in the experiments in man and the solutions were kept at room temperature and used within 20 h. Solutions were also prepared for stability testing at 23°C, and from each of these, ten duplicate samples were taken over 9 days and kept frozen (−25°C) until analysis.

Human pharmacokinetics—trial protocol

The study was approved by the Ethics Committee at the University of Lund and by the Swedish Medical Products Agency. Seven healthy male volunteers (age 24–36 years, weight 70–82 kg), who were free of medication and had no history of allergy to drugs, gave informed written consent to the study. The first subject received 125 mL of the $0{\cdot}20\,mg\,mL^{-1}$ *R*-thalidomide solution (i.e. 25 mg) infused over 60 min. From this pilot test the final

dose, expected to give a maximal blood concentration of $1-2\,\mu g\,mL^{-1}$, was decided. In a double-blind fashion six subjects received the intravenous infusion of *R*- or *S*-thalidomide into an antecubital vein on two occasions separated by at least one week. The subjects had fasted since the evening before (for at least 10 h). Using a volumetric pump, 250 mL of the drug solution (i.e. 50 mg of drug) was infused over 60 min, starting at approximately 0830 h. The subjects remained supine for the rest of the day (8–10 h), except to take a light lunch after 4 h. Blood samples were taken from an antecubital vein (opposite arm from the infusion) into heparinized tubes before and at 10, 20, 32, 45, 60, 62, 66, 70, 80, 90, 105 and 120 min and 3, 4, 5, 6, 7, 8, 10, 12, and approximately 14, 18 and 22 h after the start of the drug infusion. Each sample was immediately mixed with an equal volume of 0·025 M citrate buffer pH 1·5 and frozen (Eriksson & Björkman 1997). The blood–buffer mixtures were stored at −25°C until assay, normally for less than three days and maximally for 14 days. For both infusions to the same subject either an IMED 960 (IMED Ltd, Abingdon, England) or an IVAC 591 (IVAC Corp, San Diego, CA) volumetric infusion pump was used. The pumps were checked by authorised service personnel before the study and the deviations from the nominal volumes were found to be less than 2%.

Effect measurements

The effect measurements were performed by the same methods as previously described (Höglund et al 1998). Briefly, before each blood sampling one of the investigators noted whether the subject was awake or asleep and the subject marked his feeling of tiredness on the Borg scale. After blood sampling, a series of continuous reaction time (CRT) measurements was performed over 2 min. CRTs were measured before and at 20, 45, 70 and 90 min and 2, 3, 4, 6 and 7 h after the start of the infusion. Other possible drug effects were noted concomitantly with blood sampling.

Pharmacokinetic analysis and statistics

The central volumes of distribution (V_1 and V_3) and the rate constants for distribution, inversion and elimination were estimated by fitting the pharmacokinetic model shown in Figure 1 to the measured concentrations of *R*- and *S*-thalidomide. The model equations were derived and solved using matrix algebra and Laplace transforms (Veng-Pedersen 1978). Using observations from the four concentration curves enabled us to uniquely identify all model parameters (Heinzel et al 1977; Godfrey & Chapman 1989), as can be shown by model indistinguishability analysis. The equations were coded in the SAS software (SAS Institute, Cary, NC) and the estimations were made by the procedure NLIN. 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 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 inspections of the residuals (Eriksson et al 1995). To increase precision in the parameter estimations, all four concentration curves for each subject were fitted simultaneously.

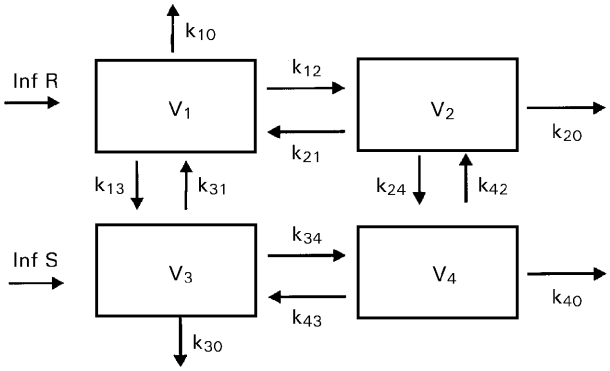

Figure 1. The model used to characterize the pharmacokinetics of *R*- and *S*-thalidomide with intravenous infusion of either enantiomer. Inf R, Inf S: drug input; k_{13}, k_{31}, k_{24}, k_{42}: rate constants for inversion; k_{12}, k_{21}, k_{34}, k_{43}: rate constants for distribution; k_{10}, k_{20}, k_{30}, k_{40}: rate constants for elimination; V_1, V_3: apparent volumes of distribution in the central compartment; V_2, V_4: apparent volumes of distribution in the peripheral compartment.

The apparent clearance of the *R*-enantiomer (CL_{appR}) was calculated as $Dose_R/AUC_R$ when this enantiomer was given and for the *S*-enantiomer as $CL_{appS} = Dose_S/AUC_S$ when this one was given. These AUCs were calculated by the logarithmic trapezoidal method with extrapolation to infinity using the fitted hybrid rate constant of the terminal elimination phase. The apparent terminal half-life ($t_{\frac{1}{2}}$) was calculated as ln(2) divided by this hybrid rate constant. Compartmental residence times for each enantiomer were calculated from the matrices of coefficients (Matis et al 1985). The system residence time of thalidomide after administration of either enantiomer (SRT_R and SRT_S) was calculated as the sum of its residence times in compartments 1–4. The real mean residence time of each (unchanged) enantiomer (MRT_R and MRT_S) was calculated as the sum of its compartmental residence times (in compartments 1–2 and 3–4, respectively).

The distributions of estimated pharmacokinetic parameters were tested by the Shapiro-Wilk statistics, setting $P = 0{\cdot}10$ as the limit for rejection of the null hypothesis of normal distribution. Normally distributed parameters are reported as mean ($\pm$ s.d.) and compared by analysis of variance. Non-normally distributed parameters are presented as median (25 and 75 percentiles). The level of significance was set at $P = 0{\cdot}05$. The SAS software was used for all statistical calculations.

Concentration–effect analysis

The methodology has been presented in detail elsewhere (Höglund et al 1998). Briefly, the natural logarithms of the observed blood concentrations of *R*-thalidomide and *S*-thalidomide were used as independent variables in a logistic regression of asleep versus not asleep, and for a series of predefined levels of tiredness on the Borg scale. These levels were: 0·5 = hardly perceptible; 1 = very light; 2 = light; 3 = moderate; 4 = tired; and 5 = very tired. In the evaluation of CRT data a threshold reaction time was estimated for each subject. The influence of *R*-thalidomide and *S*-thalidomide on threshold-subtracted CRT was investigated using a proportional hazards regression model (Cox regression) after transformation of the observed reaction times so that they exhibited a Weibull distribution appropriate for the regression. In the Cox regression function, a concentration–effect relationship is expressed as a slope of the natural logarithm of threshold-subtracted reaction time versus blood concentration of *R*- or *S*-thalidomide. These analyses were performed individually for each subject. The SAS statistical software was used for all analyses.

Simulations with the pharmacokinetic model

The blood concentration curves of *R*- and *S*-thalidomide after administration of *S*-thalidomide by various infusion schemes were simulated by means of the SAAM II software (SAAM Institute, Seattle, WA) using the pharmacokinetic model described above and values of V_1, V_3 and rate constants obtained in the six subjects.

Results

Solutions of the enantiomers of thalidomide

After 20–30 min of vortex-mixing the concentrations of *R*-, *S*- and racemic thalidomide in water and in 5% glucose solution levelled out at values corresponding to their solubilities. These were *R*-thalidomide 0·35, *S*-thalidomide 0·33 and racemate 0·070 mg mL^{-1}, in 5% glucose solution. The corresponding values in pure water were similar: 0·34, 0·33 and 0·080 mg mL^{-1}, respectively. Concentrations of the enantiomers in the 0·20 mg mL^{-1} infusion solutions were thus below saturation. After 9 days of storage in the 5% glucose solutions there was no measurable decrease in total thalidomide concentration and less than 1% of the opposite enantiomer formed.

Pharmacokinetics of the enantiomers

In the pilot study the total thalidomide concentration in the blood was 0·74 μg mL^{-1} at the end of the infusion. The concentration curves obtained with separate administration of the two enantiomers to the six subjects in the main part of the study are presented in Figure 2. After administration of the *R*-enantiomer the fitted maximum blood concentration (C_{max}) of *R*-thalidomide was $1{\cdot}20 \pm 0{\cdot}28$ μg mL^{-1} (at end of infusion) and that of formed *S*-thalidomide was $0{\cdot}16 \pm 0{\cdot}03$ μg mL^{-1} at $3{\cdot}6 \pm 0{\cdot}5$ h. After administration of the *S* enantiomer its C_{max} was $0{\cdot}93 \pm 0{\cdot}24$ μg mL^{-1} and that of formed *R*-thalidomide was $0{\cdot}23 \pm 0{\cdot}04$ μg mL^{-1} at $3{\cdot}6 \pm 0{\cdot}3$ h. The AUC values are given in Table 1. Inter-individual variations in AUC were modest, with a less than two-fold range in any value. The ratio AUC_R/AUC_S was $3{\cdot}0 \pm 0{\cdot}38$ after administration of *R*-thalidomide while the ratio AUC_S/AUC_R was only $1{\cdot}2 \pm 0{\cdot}17$ after infusion of *S*-thalidomide. The fitted and calculated pharmacokinetic parameters are shown in Table 2. A clear distinction between elimination of thalidomide from the central and peripheral compartments could not be obtained. In some subjects k_{10} (or k_{30}) was large while k_{20} (or k_{40}) was small, and in others it was the other way around. The same was true for rates of inversion.

Effect measurements

During the first 8–10 h of the study sessions, the subjects were mildly to markedly sedated or fell asleep. Induction of sedation and sleep was smooth, with no initial stimulation observed. No other adverse effects of the treatments were observed by us or reported by the subjects. Tiredness scores as functions of time are shown in Figure 3. Median tiredness scores were 3·4 at 1·1 h after infusion of *R*-thalidomide and 1·5 at 2·2 h after infusion of *S*-thalidomide.

The blood concentration of *R*-thalidomide showed significant regression with tiredness at all

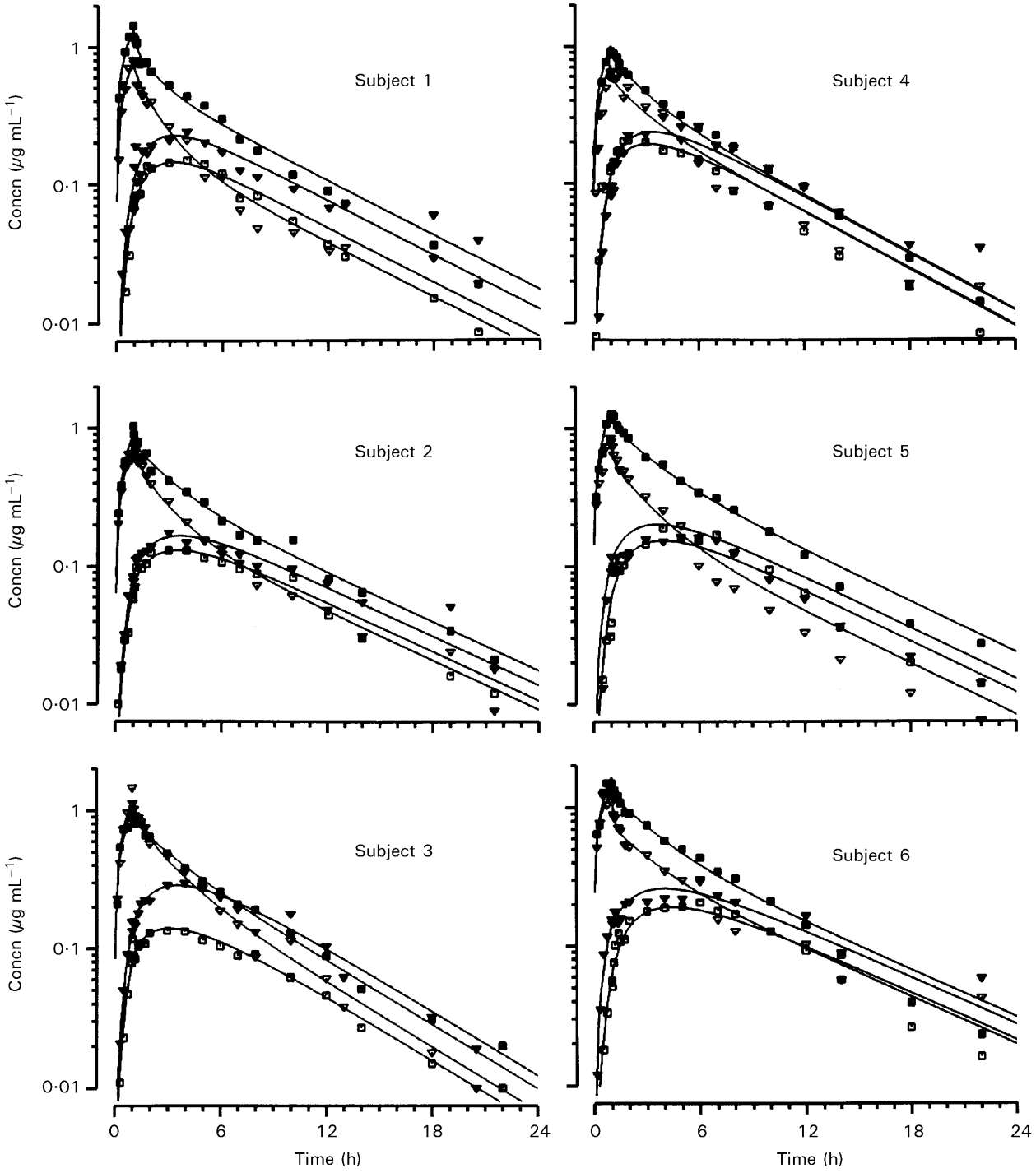

Figure 2. Observed (symbols) and fitted (curves) blood concentrations in the six subjects in the main study. In all cases, the chosen weighting factor for the fit was (predicted concentration)$^{-1}$. ■, *R*- and □, *S*-thalidomide concentrations after administration of *R*-thalidomide. ▼, *R*- and ▽, *S*-thalidomide concentrations after administration of *S*-thalidomide.

levels on the Borg scale, whereas the *S*-thalidomide concentration only attained significance as regressor at a Borg score of 1. In a reduced model, including only *R*-thalidomide concentrations, these influenced tiredness significantly at all levels. The predicted probabilities versus *R*-thalidomide concentration are given in Figure 4. The concentrations (mean and 95% confidence intervals) associated with a 50% probability were 0·11 (0·06–0·16) μg mL^{-1} for a score of 0·5 on the Borg scale, 0·22 (0·14–0·33) μg mL^{-1} for a score of 1, 0·75 (0·53–1·33) μg mL^{-1} for a score of 2 and 1·6 (1·08–3·84) μg mL^{-1} for a score of 3. By extrapolation, the mean concentration would be

Table 1. AUC ($\mu g\,h\,mL^{-1}$) values, calculated by the logarithmic trapezoidal method, for *R*- and *S*-thalidomide in blood after administration of the separate enantiomers.

	R-thalidomide given		*S*-thalidomide given	
Subject	AUC_R	AUC_S	AUC_R	AUC_S
1	4·49	1·32	2·09	2·26
2	4·06	1·47	1·99	2·59
3	4·27	1·34	2·81	3·93
4	4·27	1·74	2·74	2·84
5	5·88	1·88	1·92	2·65
6	7·15	2·51	3·35	4·52
Median	4·38	1·61	2·42	2·75
25; 75 percentiles	4·27; 5·88	1·34; 1·88	1·99; 2·81	2·59; 3·93

$2{\cdot}3\,\mu g\,mL^{-1}$ for a score of 4 and $3{\cdot}5\,\mu g\,mL^{-1}$ for a score of 5.

The probability of sleep was marginally significantly ($P = 0{\cdot}057$) correlated with the blood concentration of *R*-thalidomide but not with that of *S*-thalidomide ($P = 0{\cdot}50$) when both concentrations were included as regressors. In a stepwise logistic regression *R*-thalidomide concentration, but not *S*-thalidomide concentration, was identified as a significant regressor. After deletion of the latter from the model, the probability of sleep was significantly ($P = 0{\cdot}0052$) correlated with the concentration of *R*-thalidomide. The observations, together with the predicted probability versus *R*-thalidomide blood concentration are shown in Figure 5. The predicted concentration (mean and 95% confidence limits) associated with a 20% probability of sleep was 0·77 ($0{\cdot}41-3{\cdot}96$) $\mu g\,mL^{-1}$.

The infusions had no clear effects on CRT, as shown in Figure 6. The estimate of the threshold reaction times was 125 ± 17 ms. In the Cox regression model for ln(reaction time minus

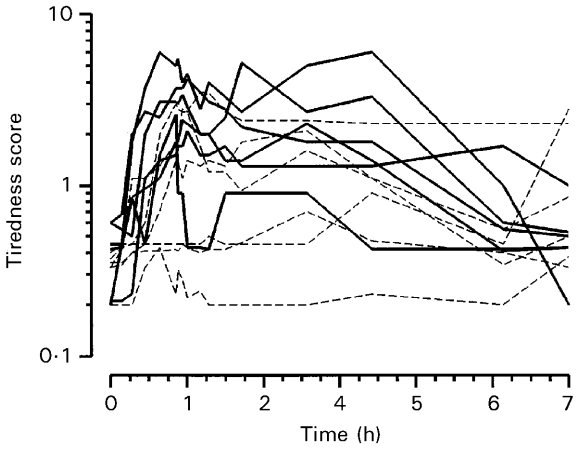

Figure 3. Tiredness scores as functions of time during and after infusion of *R*-thalidomide (——) or *S*-thalidomide (- - -) to 6 subjects.

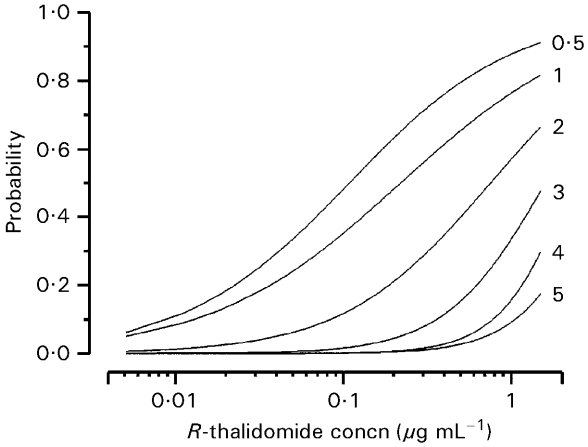

Figure 4. Predicted probabilities, obtained by logistic regression, of attaining at least a certain level of tiredness on the Borg scale vs *R*-thalidomide blood concentration. The levels of tiredness are: 0·5, hardly perceptible; 1, very light; 2, light; 3, moderate; 4, tired; 5, very tired.

Table 2. Fitted and calculated pharmacokinetic parameters of thalidomide.

		Enantiomer		
Parameter[a]	Unit	*R*-thalidomide	*S*-thalidomide	*P* value (if tested)
V_1; V_3†	L	$18 \pm 7{\cdot}5$	24 ± 11	
k_{10}; k_{30}	h^{-1}	$0{\cdot}29 \pm 0{\cdot}17$	$0{\cdot}31 \pm 0{\cdot}20$	
k_{20}; k_{40}	h^{-1}	0·014 (0·0012; 0·028)	$0{\cdot}14 \pm 0{\cdot}13$	
k_{12}; k_{34}	h^{-1}	$5{\cdot}5 \pm 2{\cdot}7$	$4{\cdot}4 \pm 3{\cdot}0$	
k_{21}; k_{43}	h^{-1}	$2{\cdot}1 \pm 1{\cdot}0$	$1{\cdot}9 \pm 0{\cdot}8$	
k_{13}; k_{31}	h^{-1}	0·24 (0·14; 0·29)	$0{\cdot}34 \pm 0{\cdot}11$	
k_{24}; k_{42}	h^{-1}	$0{\cdot}094 \pm 0{\cdot}086$	$0{\cdot}12 \pm 0{\cdot}07$	
CL_{appR}; CL_{appS}	L/h	$10 \pm 2{\cdot}1$	$21 \pm 4{\cdot}6$	$< 0{\cdot}05$
SRT_R; SRT_S	h	$7{\cdot}0 \pm 1{\cdot}1$	$6{\cdot}3 \pm 0{\cdot}7$	0·135
MRT_R; MRT_S	h	$4{\cdot}7 \pm 0{\cdot}7$	$3{\cdot}9 \pm 0{\cdot}6$	0·063
Terminal $t_{\frac{1}{2}}$	h	$4{\cdot}7 \pm 0{\cdot}5$	$4{\cdot}7 \pm 0{\cdot}5$	

Normally distributed parameters are presented as mean ± s.d. and non-normally distributed parameters as median and 25; 75 percentiles. [a]The first parameter (e.g., V_1) refers to *R*-thalidomide and the second one (e.g. V_3) to *S*-thalidomide.

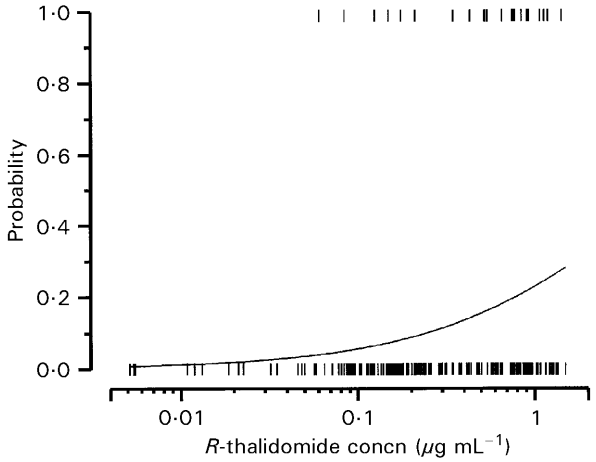

Figure 5. Observed (bars) sleep (probability = 1) vs not asleep (probability = 0) and predicted probability (curve) of sleep vs *R*-thalidomide blood concentration, obtained by logistic regression.

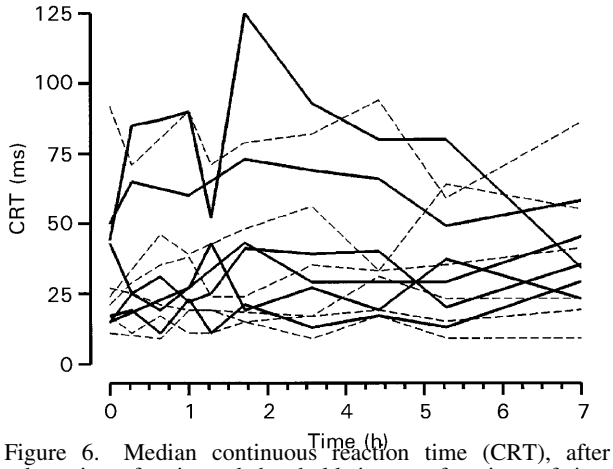

Figure 6. Median continuous reaction time (CRT), after subtraction of estimated threshold time, as functions of time during and after infusion of *R*-thalidomide (——) or *S*-thalidomide (- - -) to 6 subjects.

threshold), where both the *R*- and *S*-thalidomide concentrations were incorporated as regressors, the median slope for the *R* enantiomer showed a trend towards a significant difference from zero ($P = 0{\cdot}094$), whereas the slope for the *S*-thalidomide concentration did not ($P = 0{\cdot}44$). In the model where only the *R*-thalidomide concentration was incorporated as a regressor, its median slope was 0·40 ln(ms) × $(\mu g\,mL^{-1})^{-1}$, but significance was still not attained ($P = 0{\cdot}094$).

Simulations with the pharmacokinetic model

Predicted blood concentration curves after administration of 50 mg *S*-thalidomide over 10 min are shown in Figure 7. The C_{max} of infused *S* enantiomer varied between 1·2 and 3·4 $\mu g\,mL^{-1}$,

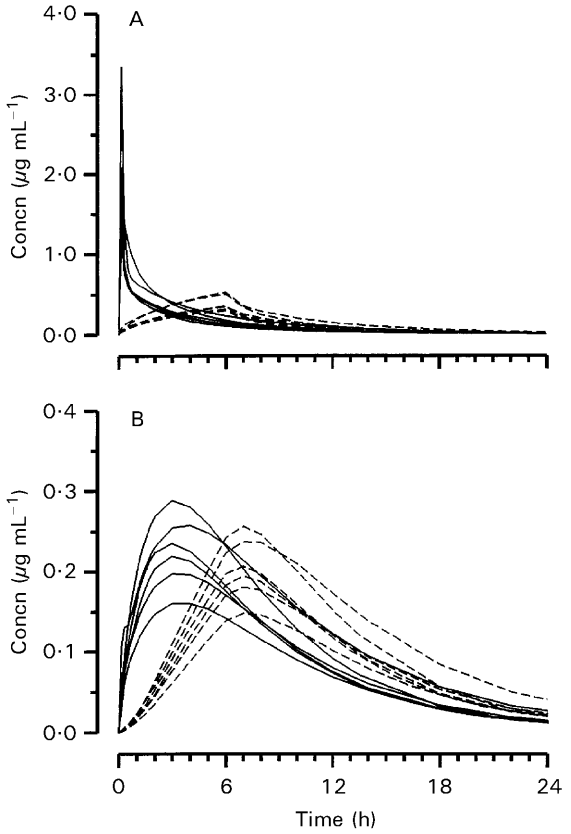

Figure 7. Predicted blood concentration curves of (A) *S*- and (B) *R*-thalidomide in the 6 subjects during and after intravenous infusions of 50 mg of *S*-thalidomide over either 10 min (——) or 6 h (- - -). Note the 10-fold difference in scale between the concentration axes.

while that of formed *R* enantiomer was $0{\cdot}23 \pm 0{\cdot}05\,\mu g\,mL^{-1}$ at 3–3·5 h (i.e. very similar to C_{max} after the 1-h infusion of *S*-thalidomide). Predicted blood concentration curves after giving a dose of 50 mg *S*-thalidomide over 6 h are also shown in Figure 7. Now the C_{max} of infused *S* enantiomer varied between 0·30 and 0·53 $\mu g\,mL^{-1}$, while that of formed *R* enantiomer was $0{\cdot}20 \pm 0{\cdot}04\,\mu g\,mL^{-1}$ at around 7 h. Variations between subjects were small despite the apparent differences in rates of peripherally and centrally occurring processes. Changing the rate of infusion of *S*-thalidomide thus had little influence on predicted C_{max} of *R*-thalidomide.

Discussion

Preparation of intravenous formulations of racemic thalidomide is not feasible due to the low solubility of this compound. However, the separate enantiomers have been reported to have a five times higher solubility than the racemate (Hague & Smith 1988; Krenn et al 1992). Therefore, we judged that it should be possible to prepare, at least, rather dilute

intravenous formulations of *R*- and *S*-thalidomide. Adequate chemical stability was achieved at the normal pH of 4–5 of a 5% glucose solution.

To our knowledge, thalidomide has thus not previously been given intravenously to man. For safety reasons we gave the enantiomers as an infusion over 1 h, aiming at the maximum total thalidomide concentration of $1–2\,\mu g\,mL^{-1}$ observed after an oral dose of $1{\cdot}5\,mg\,kg^{-1}$ (Eriksson et al 1995). Based on approximate estimates of the volume of distribution from previous studies on thalidomide (Chen et al 1989; Eriksson et al 1995), we calculated that a dose of 50 mg might be appropriate. For safety reasons we gave a test dose of 25 mg to the first subject and since the obtained blood concentration data confirmed our estimation, the final dose was decided as 50 mg.

The study of the pharmacokinetics of thalidomide has also been hampered by the lack of an intravenous formulation. It has, however, been established that extrahepatic spontaneous hydrolysis is the main mechanism of elimination of thalidomide in-vivo, with hepatic metabolism and renal excretion playing minor roles (Schumacher et al 1965; Chen et al 1989). The apparent pharmacokinetics of racemic thalidomide has been studied after oral administration to healthy volunteers (Chen et al 1989; Trapnell et al 1998) and to patients with HIV infection or cancer (Piscitelli et al 1997; Figg et al 1999; Noormohamed et al 1999). Since the non-stereospecific HPLC assays only measured the sum of the plasma concentrations of *R*- and *S*-thalidomide, and also since pharmacokinetic parameters were either calculated arbitrarily assuming complete bioavailability (F), or expressed as divided by F, the results did not describe the pharmacokinetics of the enantiomers of thalidomide. Using a stereospecific assay, we (Eriksson et al 1995) investigated the disposition of the enantiomers of thalidomide after oral administration to men. This study confirms our previous findings that the enantiomers of thalidomide undergo rapid chiral inversion in-vivo and that *R*-thalidomide is the favoured enantiomer as regards AUC and blood concentration at pseudoequilibrium.

Previously (Eriksson et al 1995) we noted that a mean oral dose of 80 mg of *R*-thalidomide gave a mean AUC_R of $8{\cdot}59\,\mu g\,h\,mL^{-1}$ (mean AUC/dose $= 0{\cdot}11\,h\,L^{-1}$), while here an intravenous dose of 50 mg gave $AUC_R = 5{\cdot}02\,\mu g\,h\,mL^{-1}$ (AUC/dose $= 0{\cdot}10\,h\,L^{-1}$). Similarly for *S*-thalidomide, mean AUC/dose was $0{\cdot}049\,h\,L^{-1}$ after oral and $0{\cdot}063\,h\,L^{-1}$ after intravenous administration. Even though comparison with historical data cannot be regarded as a proper study, this suggests that the mean oral bioavailability of thalidomide is high (as previously discussed in Eriksson et al (1995), bioavailability of the *R*-enantiomer could theoretically exceed 100% when the racemate is given).

When reversible biotransformation occurs, calculation of the clearance of a drug by the standard formula of dose/AUC yields an underestimation of the true clearance (Ebling & Jusko 1986). This is because some of the drug that has been cleared reversibly, in this case by inversion, will return to the circulation. Similarly, calculation of an apparent V_{ss} from the AUC and the area under the first moment curve of plasma concentrations will give an overestimation.

Despite this, the apparent CL values for *R*- and *S*-thalidomide are given in Table 2 since they can be used to calculate steady-state concentrations of the enantiomers during a long-term infusion. Showing apparent V_{ss} values would, on the other hand, be meaningless.

Methods have been described for calculating realistic estimates of clearance, volume and residence-time parameters for compounds with reversible kinetics (Ebling & Jusko 1986). However, these methods assume that drug distributed to peripheral compartments does not undergo elimination or interconversion. For the enantiomers of thalidomide, this assumption is unrealistic. In-vitro experiments (Knoche & Blaschke 1994; Eriksson et al 1995, 1998; Reist et al 1998) indicate that the enantiomers undergo non-enzymic hydrolysis and inversion in any aqueous medium at physiological temperature and pH. Based on this knowledge and on the chosen mode of drug administration, we constructed the simplest biologically plausible model, in which two two-compartment models were connected by rate constants for chiral inversion between both the central and the peripheral compartments and elimination occurs from all compartments. This model yielded values for all parameters needed for simulation of various dosage schedules (i.e. the volumes of the two central compartments and all rate constants).

The true MRT of a drug with reversible biotransformation is the sum of the compartmental residence times of the unchanged molecule (i.e. for *R*-thalidomide in $V_1 + V_2$ and for *S*-thalidomide in $V_3 + V_4$). The SRT, on the other hand, includes also the time that each molecular species has spent as the opposite enantiomer before being eliminated (SRT is the sum of compartmental residence times in all compartments). SRT will, however, depend on which enantiomer was originally administered, thus SRT_R and SRT_S are different. From the values in Table 2 one may deduce that a given enantiomer of thalidomide spends approximately one-third of its time in the system inverted to the opposite species.

There was excellent agreement between this study and our earlier study (Eriksson et al 1995) as regards fitted terminal half-lives. When equilibrium between the enantiomers has been established, their apparent terminal half-lives must be identical, and the mean value was 4·7 h in both studies. Initially an apparent elimination half-life of $8{\cdot}7 \pm 4{\cdot}1$ h was reported (Chen et al 1989). However, in that study, precautions against ex-vivo hydrolysis of thalidomide in the blood samples were not taken and, more importantly, samples were taken frequently over 12 h, followed by only a single sample after 24 h. Thus, the terminal half-life was estimated on only a few concentration values in the elimination phase. The resulting wide range of values (3·0–14·6 h) presumably reflects experimental uncertainty more than actual inter-individual variation. With better sampling schedules, the overall mean half-life of thalidomide was later estimated at 6·2 h (range, 3·7–11·5 h) (Piscitelli et al 1997), $6{\cdot}7 \pm 1{\cdot}7$ h (Trapnell et al 1998), $7{\cdot}1 \pm 1{\cdot}9$ h (Figg et al 1999) or $4{\cdot}4 \pm 1{\cdot}3$ to $5{\cdot}5 \pm 2{\cdot}5$ h (Noormohamed et al 1999). After oral administration, estimation of elimination half-life can also be confounded by slow absorption of poorly soluble thalidomide from the gastrointestinal tract. In this study, where absorption was not a problem, the range of half-lives was only 3·9–5·3 h, in agreement with the modest inter-individual variability of AUC and hence of apparent and real clearance. This is conceivably due to the main mechanism of elimination of thalidomide. There is little variability in rates of hydrolysis and inversion in the well-controlled environment (pH, temperature and plasma albumin concentration) of the human body.

Another consequence of the fact that hydrolysis is the main route of elimination is that thalidomide should not induce or inhibit its own elimination. This has also recently been demonstrated in multidose pharmacokinetic studies on orally administered thalidomide (Trapnell et al 1998; Figg et al 1999).

The findings in this study confirm our previous observation (Höglund et al 1998) that sedation and sleep relate to blood concentrations of *R*- but not *S*-thalidomide. A corresponding trend was observed in the CRT data, although in this study statistical significance was not attained. Generally, the sedative potency of *R*-thalidomide appeared to be lower in the present than in the previous study. In the current study the concentration of *R*-thalidomide associated with a 20% probability of sleep was $0{\cdot}77\ \mu g\,mL^{-1}$, as compared with $0.13\ \mu g\,mL^{-1}$ previously. The observed effects of *R*-thalidomide on tiredness also differed between the studies, albeit less than that on sleep. The concentration associated with a 50% probability for a tiredness score of 3 was $1{\cdot}6\ \mu g\,mL^{-1}$ in the present and $0{\cdot}53\ \mu g\,mL^{-1}$ in the earlier study. Finally, the median slope relating prolongation of CRT to blood concentration of *R*-thalidomide was 0·40 ln (ms) × $(\mu g\,mL^{-1})^{-1}$ in this study as compared with individual values of 0·48–1·64 in the previous study.

In the previous study (Höglund et al 1998) we observed that subjects were often awake and alert at 5 h after a dose (i.e., directly after lunch) irrespective of treatment and drug concentrations. The most important explanation for the discrepant results is, therefore, probably the differences in the experimental setting; in this study there was a high level of activity around the subjects during the period of infusion when blood concentrations of the thalidomide enantiomers were high. It thus appears that the sedative effect of *R*-thalidomide is rather dependent on situation. From the available data it cannot, however, be excluded that oral administration of thalidomide does cause more sedation than intravenous infusion achieving similar blood concentrations of *R*-thalidomide. This would then suggest that some sedation was due to an active metabolite formed during the first passage through the liver or gut mucosa. Such a hypothesis is highly speculative. From a clinical point of view it seems safe to conclude that intravenous infusion of the enantiomers of thalidomide should at least not cause more sedation than oral administration giving similar blood concentrations of *R*-thalidomide.

It should also be mentioned that CRT measurements in the previous study were performed over 10 min. Since changes in blood concentrations of *R*-thalidomide were expected to occur more rapidly after intravenous infusion than after oral dosing a 2-min measurement period was chosen in this study to avoid the problem of large changes in concentration during a measurement. However, comparing the first 2 min of sampling with the full 10 min in our previous dataset of 297 10-min measurements revealed no significant differences in final estimates of concentration–effect relationships. This is in contrast to a similar comparison of CRT data obtained after administration of morphine, where a successive prolongation of CRT during the 10-min period was found (Westerling et al 1993; Höglund, unpublished data).

In conclusion, we prepared and gave intravenous infusions of the enantiomers of thalidomide for the first time. The infusion solutions could be used for clinical purposes if aspects of solubility, stability and possible toxicity are carefully considered. No toxicity was observed, apart from the expected sedative effects. The disposition of each enantiomer could be described by means of a

compartmental model allowing prediction of blood concentration curves after alternative infusion schemes. The study confirmed a previous finding that the sedative effects were significantly correlated with blood concentrations of *R*-thalidomide but not with those of *S*-thalidomide. Predictions by the pharmacokinetic model suggested that varying the infusion time of a fixed dose of the presumably immunomodulating *S*-thalidomide between 10 min and 6 h had little influence on the maximal attained concentration of the sedative *R*-enantiomer.

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References

Atra, E., Sato, E. I. (1993) Treatment of the cutaneous lesions of systemic lupus erythematosus with thalidomide. Clin. Exp. Rheumatol. 11: 487–493

Boughton, B. J., Sheehan, T. M. T., Wood, J., O'Brien, D., Butler, M., Simpson, A., Hale, K. A. (1995) High-performance liquid chromatographic assay of plasma thalidomide: stabilization of specimens and determination of tentative therapeutic range for chronic graft-versus-host disease. Ann. Clin. Biochem. 32: 79–83

Chen, T.-L., Vogelsang, G. B., Petty, B. G., Brundrett, R. B., Noe, D. A., Santos, G. W., Colvin, O. M. (1989) Plasma pharmacokinetics and urinary excretion of thalidomide after oral dosing in healthy male volunteers. Drug Metabol. Disp. 17: 402–405

Ebling, W. F., Jusko, W. J. (1986) The determination of essential clearance, volume, and residence time parameters of recirculating metabolic systems: the reversible metabolism of methylprednisolone and methylprednisone in rabbits. J. Pharmacokin. Biopharm. 14: 557–599

Eriksson, T., Björkman, S. (1997) Handling of blood samples for determination of thalidomide. Clin. Chem. 43: 1094–1095

Eriksson, T., Björkman, S., Fyge, Å., Ekberg, H. (1992) Determination of thalidomide in plasma and blood by high-performance liquid chromatography: avoiding hydrolytic degradation. J. Chromatogr. 582: 211–216

Eriksson, T., Björkman, S., Roth, B., Fyge, Å., Höglund, P. (1995) Stereospecific determination, chiral inversion in vitro and pharmacokinetics in humans of the enantiomers of thalidomide. Chirality 7: 44–52

Eriksson, T., Björkman, S., Roth, B., Fyge, Å., Höglund, P. (1998) The enantiomers of thalidomide: blood distribution and the influence of serum albumin on chiral inversion and hydrolysis. Chirality 10: 223–228

Figg, W. D., Raje, S., Bauer, K. S., Tompkins, A., Venzon, D., Bergan, R., Chen, A., Hamilton, M., Pluda, J., Reed, E. (1999) Pharmacokinetics of thalidomide in an elderly prostate cancer population. J. Pharm. Sci. 88: 121–125

Godfrey, K. R., Chapman, M. J. (1989) The problem of model indistinguishability in pharmacokinetics. J. Pharmacokin. Biopharm. 17: 229–267

Hague, D., Smith, R. L. (1988) Enigmatic properties of (±)-thalidomide; an example of a stable racemic compound. Br. J. Clin. Pharmacol. 26: 623P

Hamuryudan, V., Mat, C., Saip, S., Özyazgan, Y., Siva, A., Yurdakul, S., Zwingenberger, K., Yazici, H. (1998) Thalidomide in the treatment of mucocutaneous lesions of the Behçet syndrome. Ann. Intern. Med. 128: 443–495

Heinzel, G., Wolf, V. G., Koss, F. W., Bozler, G. (1977) Modellentwicklung in der Pharmakokinetik. IV. Teil: Die Ermittlung der Mikrokonstanten in linearen Kompartmentmodellen. Arzneim. Forsch. 27: 912–931

Heney, D., Norfolk, D. R., Wheeldon, J., Bailey, C. C. Lewis, I. J., Barnard, D. L. (1991) Thalidomide treatment for chronic graft-versus-host disease. Br. J. Haematol. 78: 23–27

Höglund, P., Eriksson, T., Björkman, S. (1998) A double-blind study of the sedative effects of the thalidomide enantiomers in humans. J. Pharmacokin. Biopharm. 26: 363–383

Jacobson, J. M. Greenspan, J. S., Spritzler, J., Ketter, N., Fahey, J. L., Jackson, J. B., Fox, L., Chernoff, M., Wu, A. W., MacPhail, L. A., Vasquez, G. J., Wohl, D. A. (1997) Thalidomide for the treatment of oral aphthous ulcers in patients with human immunodeficiency virus infection. N. Engl. J. Med. 336: 1487–1493

Jacobson, J. M., Spritzler, J., Fox, L., Fahey, J. L., Jackson, J. B., Chernoff, M., Wohl, D. A., Wu, A. W., Hooton, T. M., Sha, B. E., Shikuma, C. M., MacPhail, L. A., Simpson, D. M., Trapnell, C. B., Basgoz, N. (1999) Thalidomide for the treatment of esophageal aphtous ulcers in patients with human immunodeficiency virus infection. J. Infect. Dis. 180: 61–67

Knoche, B., Blaschke, G. (1994) Investigations on the in vitro racemization of thalidomide by HPLC. J. Chromatogr. A 666: 235–240

Koch, H. P. (1984) Thalidomide and congeners as anti-inflammatory agents. Prog. Medicinal Chem. 22: 165–242

Krenn, M. Gamcsik, M. P., Vogelsang, G. B. Colvin, O. M., Leong, K. W. (1992) Improvements in solubility and stability of thalidomide upon complexation with hydroxypropyl-β-cyclodextrin. J. Pharm. Sci. 81: 685–689

Matis, J. H., Wehrly, T. E., Gerald, K. B. (1985) Use of residence time moments in compartmental analysis. Am. J. Physiol. 249: E409–E415

Nightingale, S. L. (1998) Thalidomide approved for erythema nodosum leprosum. J. Am. Med. Assoc. 280: 872

Nishimura, K., Hashimoto, Y., Iwasaki, S. (1994) (*S*)-form of α-methyl-N(α)-phthalimido-glutarimide, but not its (*R*)-form, enhances phorbol ester-induced tumour necrosis factor-α production by human leukaemia cell HL-60: implication of optical resolution of thalidomidal effects. Chem. Pharm. Bull. 42: 1157–1159

Noormohamed, F. H., Youle, M. S., Higgs, C. J., Kook, K. A., Hawkins, D. A., Lant, A. F., Thomas, S. D. (1999) Pharmacokinetics and hemodynamic effects of single oral doses of thalidomide in asymptomatic human immunodeficiency virus-infected subjects. Aids Res. Hum. Retroviruses 15: 1047–1052

Piscitelli, S. C., Figg, W. D., Hahn, B., Kelly, G., Thomas, S., Walker, R. E. (1997) Single-dose pharmacokinetics of thalidomide in human immunodeficiency virus-infected patients. Antimicrob. Agents Chemother. 41: 2797–2799

Reist, M., Carrupt, P.-A., Francotte, E., Testa, B. (1998) Chiral inversion and hydrolysis of thalidomide: mechanisms and catalysis by bases and serum albumin, and chiral stability of teratogenic metabolites. Chem. Res. Toxicol. 11: 1521–1528

Schmidt, H., Rush, B., Simonian, G., Murphy, T., Hsieh, J., Condon, M. (1996) Thalidomide inhibits TNF response and increases survival following endotoxin injection in rats. J. Surg. Res. 63: 143–146

Schuler, S., Ehninger, U. (1995) Thalidomide: rationale for renewed use in immunological disorders. Drug Safety 12: 364–369

Schumacher, H., Smith, R. L., Williams, R. T. (1965) The metabolism of thalidomide: the spontaneous hydrolysis of thalidomide in solution. Br. J. Pharmacol. 25: 324–337

Singhal, S., Mehta, J., Desikan, R., Ayers, D., Roberson, P., Eddlemon, P., Munshi, N., Anaissie, E., Wilson, C., Dhodapkar, M., Zeldis, J., Barlogie, B. (1999)Antitumor activity of thalidomide in refractory multiple myeloma. N. Eng. J. Med. 341: 1565–1571

Trapnell, C. B., Donahue, J. M. Collins, S. R., Flockhart, D. A., Thacker, D., Abernethy, D. R. (1998) Thalidomide does not alter the pharmacokinetics of ethinyl estradiol and norethindrone. Clin. Pharmacol. Ther. 64: 597–602

Veng-Pedersen, P. (1978) General treatment of linear pharmacokinetics. J. Pharm. Sci. 67: 187–191

Westerling, D., Frigren, L., Höglund, P. (1993) Morphine pharmacokinetics and effects on salivation and continuous reaction times in healthy volunteers. Ther. Drug Monit. 15: 364–374

Wnendt, S., Finkam, M., Winter, W., Ossig, J., Raabe, G., Zwingenberger, K. (1996) Enantioselective inhibition of TNF-α release by thalidomide and thalidomide-analogues. Chirality 8: 390–396

Vogelsang, G. B., Farmer, E. R., Hess, A. D., Altamonte, V., Beschorner, W. E., Jabs, D. A., Corio, R. L., Levin, L. S., Colvin, O. M., Wingard, J. R., Santos, G. W. (1992) Thalidomide for the treatment of chronic graft-versus-host disease. N. Engl. J. Med. 326: 1055–1058

Zwingenberger, K., Wnendt, S. (1996) Immunomodulation by thalidomide: systemic review of the literature and of unpublished observations. J. Inflamm. 46: 177–211