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Hydroxylated Metabolites of Thalidomide: Formation In-vitro and In-vivo in Man

TOMMY ERIKSSON, SVEN BJÖRKMAN, BODIL ROTH, HENRIK BJÖRK* AND PETER HÖGLUND*

*Hospital Pharmacy, Malmö University Hospital and *Department of Clinical Pharmacology, Lund University, Sweden*

Abstract

There is renewed interest in the clinical use of thalidomide, because of its unique immunomodulating action. Because data on the metabolism of thalidomide in man are very sparse, the aim of this study was to develop HPLC assays for the 5-hydroxy, 5,6-dihydroxy, 4,5-dihydroxy and 5′-hydroxy metabolites of thalidomide and to investigate their possible formation in man—in-vitro in liver homogenates and in-vivo in healthy volunteers.

Reversed-phase HPLC assays with UV detection were developed for quantification of the metabolites in the low ng mL^{-1} range in plasma and incubate samples. The stability of the metabolites was investigated and degradation was avoided by rapid chilling and acidification of the samples. After incubation of thalidomide with fraction S9 from human liver, formation of the 5-hydroxy and 5′-hydroxy metabolites could be demonstrated. The 5′-hydroxy metabolite was found, in low concentrations, in plasma samples from eight healthy male volunteers who had received thalidomide orally. The other three metabolites could not be found by HPLC with detection limits of 1–2 ng mL^{-1}.

Thus the formation of two hydroxylated metabolites of thalidomide in the liver in man was demonstrated, but only one of these could be detected in the circulation.

Thalidomide (α-phthalimidoglutarimide, Table 1) has a chiral center and occurs as a racemate of the *R*-(+) and *S*-(−) enantiomers. The racemate was used in human therapy as a sedative, but was withdrawn mainly because of its teratogenic effects (Koch 1985). Because of its immunomodulating effects there is a growing clinical interest in the use of thalidomide in inflammatory and immunological disorders (Schuler & Ehninger 1995; Zwingenberger & Wnendt 1996). Last year the US Food and Drug Administration announced its decision to approve thalidomide for the treatment of erythema nodosum leprosum and approval for other indications might follow (Marwick 1997).

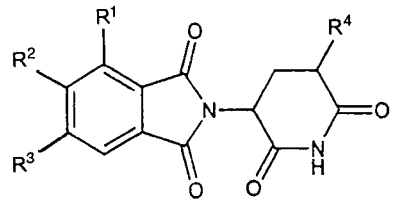
The molecular mechanism of the effects of thalidomide remains unclear and the possible role of active metabolites has not been elucidated (Günzler 1992; Zwingenberger & Wnendt 1996; Neubert & Neubert 1997). Thalidomide is hydrolysed in-vivo and in-vitro to twelve different products (Schumacher et al 1965a), eleven of which retain the chiral center. Hydroxylation is possible at five different carbon atoms and on two of these this will create new chiral centres (Schumacher et al 1965b). The metabolites can then in turn be degraded by hydrolysis. In addition, arene oxides (Gordon et al 1981), an *N*-hydroxy metabolite (Blaschke et al 1989) and possibly conjugates could be formed. The number of possible metabolites and degradation products of racemic thalidomide thus well exceeds 100 (Schumacher et al 1965b).

A metabolite of thalidomide with a hydroxy function at the 4-position was tentatively identified in human urine more than 30 years ago (Smith et al 1962). Apart from this, there are no published studies on its metabolism in man. It has been demonstrated that elimination of thalidomide in-vivo is chiefly a result of spontaneous hydrolysis whereas renal excretion and enzymic metabolism presumably play minor roles (Schumacher et al 1965a; Chen et al 1989).

The purpose of this work was to develop HPLC assays for 5-hydroxy-, 5,6-dihydroxy-, 4,5-dihydroxy- and 5′-hydroxythalidomide (Table 1) and to study the possible formation of these metabolites in

Correspondence: Sven Björkman, Hospital Pharmacy, Malmö University Hospital, S-205 02 Malmö, Sweden.

Table 1. The structures of thalidomide and its hydroxylated metabolites investigated in this study.



Compound	R^1	R^2	R^3	R^4
Thalidomide	H	H	H	H
5-Hydroxythalidomide	H	OH	H	H
4,5-Dihydroxythalidomide	OH	OH	H	H
5,6-Dihydroxythalidomide	H	OH	OH	H
5′-Hydroxythalidomide	H	H	H	OH

EM 12, used as an internal standard, is the monooxoisoindolyl analogue of thalidomide.

man—in-vitro in liver homogenates and in-vivo in healthy male volunteers, during exposure to *R*-, *S*- or racemic thalidomide.

Materials and Methods

Thalidomide racemate and enantiomers, 5-hydroxythalidomide and EM 12 [3-(1,3-dihydro-1-oxo-2*H*-isoindol-2-yl)-2,6-dioxopiperidine] were kindly supplied by Grünenthal (Stolberg/Rheinland, Germany), 5′-hydroxy- (Teubert et al 1998), 4,5-dihydroxy- and 5,6-dihydroxyhalidomide were gifts from Professor Kurt Eger and Dr Uwe Teubert (Institut für Pharmazie und Pharmazeutische Chemie, Leipzig, Germany), and phenacetin was of European Pharmacopoeia quality. Human liver S9 fraction (Pooled HepatoSNine) 20 mg mL^{-1} was purchased from Human Biologics (Phoenix, AZ) and fraction S9 rabbit liver homogenate (Östraat et al 1996) was a gift from Dr Kristian Riesbeck (Department of Medical Microbiology, Malmö University Hospital, Sweden). All stock solutions were prepared in methanol and kept at −25°C except for EM 12 and phenacetin which were kept at 4°C.

HPLC assays

All biological samples, plasma, blood or liver incubates, were immediately mixed with 25 mM citrate buffer (pH 1·5), frozen as soon as possible and stored at −25°C (Eriksson & Björkman 1997). The volume relationship between sample and buffer was 2 : 1 for plasma and 1 : 1 for all other samples. HPLC was performed with an LDC/Milton Roy (Riviera Beach, FL) Constametric III pump, a Rheodyne 7125 loop injector with a 20-μL loop, a Spectromonitor III variable-wavelength UV-detector and a 250 mm × 4 mm i.d., 7 μm particle size, LiChrocart RP-18 column (Merck). Chromatographic conditions, precision, stability in stock solutions and in blood samples, sample work-up, and extraction yields have previously been reported for racemic thalidomide and for the separate enantiomers (Eriksson et al 1992, 1995; Eriksson & Björkman 1997). Chromatographic conditions for determination of the four metabolites are shown in Table 2. In addition, extracts of liver homogenates and plasma samples with chromatographic peaks corresponding to metabolites were analysed by use of a Waters (Milford, MA) model 996 photodiode-array detector. They were compared with extracts of blank liver incubation mixture or blank plasma to which comparable amounts (10–240 ng) of the synthetic metabolites had been added. UV spectra corresponding to the peaks of interest were compared by use of Millennium 2.1 software (Waters).

Table 2. Characteristics of the HPLC assays used for the metabolites of thalidomide.

	5-Hydroxy	5,6-Dihydroxy	4,5-Dihydroxy	5′-Hydroxy
Mobile phase (acetonitrile–buffer)*	25 : 75	17 : 83	13·5 : 86·5	13·5 : 86·5
pH	3	3	3·5	3·5
Flow rate (mL min^{-1})	1·2	1·8	1·5	1·5
Detection wavelength (nm)	235	252	227	227
Capacity factor (k′)	2·5	2·9	5	6
Limit of detection in plasma (ng mL^{-1})	1	1	2	2
CV at 10·0 ng (%)	6·4	6·4	8·5	7·1
Amount found (mean ± s.d., ng)	9·8 ± 0·6	9·9 ± 0·6	11·9 ± 1·0	11·6 ± 0·8
CV at 20·0 ng (%)			3·8	8·8
Amount found (mean ± s.d., ng)			20·6 ± 0·8	18·7 ± 1·6
Extraction yield (mean ± s.d., %)	98 ± 7·3	88 ± 6·9	57 ± 6·5	89 ± 7·0

*0·1 M Phosphate buffer. For the 4,5-dihydroxy and 5′-hydroxy metabolites 20 mM triethylamine was added.

Sample preparation

5-Hydroxy- and 5,6-dihydroxy-thalidomide. To each sample (normally 1·5 mL plasma–buffer mixture or 0·8–1·2 mL liver homogenate–buffer mixture) were added internal standard solution (5 $\mu g\,mL^{-1}$ phenacetin; 30 μL) and NaCl (approx. 0·1 g). The samples were extracted with diethyl ether (2 × 5·0 mL) by vortex-mixing for 45 s and centrifuged at 1200 *g* for 10 min. The combined organic phases were evaporated under a stream of dry air, the residue was dissolved in 50 μL mobile phase and 20 μL of this solution was injected into the chromatograph.

4,5-Dihydroxy- and 5′-hydroxythalidomide As above, but the internal standard was EM 12 solution (50 $\mu g\,mL^{-1}$, normally 30 μL), phosphate buffer (0·1 M, 0·5 mL) containing 20 mM triethylamine (pH 3·5) was added, and extraction was performed once with ethyl acetate (5 mL).

Analysis Standard curves were prepared from plasma–buffer (2 : 1, 1·5 mL) to which internal standard and 5–40 (or 10–80) ng 5-hydroxy-, 4,5-dihydroxy-, 5,6-dihydroxy- or 5′-hydroxythalidomide had been added. For stability tests standard curves with 5–10 times higher amounts were used. Precision in plasma–buffer samples was checked by assaying eight samples (1·5 mL) to which 10 or 20 ng metabolite had been added. Limits of detection, defined as a peak-to-noise ratio of 3, were estimated. The extraction yields from plasma–buffer were determined by use of 3–5 samples (1·0 mL), each containing 100 ng 5-hydroxy- and 5,6-dihydroxythalidomide. After sample preparation phenacetin (150 ng) was added as external standard for quantitation. The extraction yield for 4,5-dihydroxy- and 5′-hydroxythalidomide were determined analogously, using EM 12 (500 ng) as internal standard. The extraction yield for EM 12 was also determined, using 1·5 μg EM 12 and 400 ng 5′-hydroxythalidomide as standards.

Because of the risk of interference in the chromatograms, possible sources of contaminants, e.g. plastic laboratory ware, detergents and tap water, were avoided. Glass-stoppered tubes washed with distilled water and with 99% ethanol were used throughout.

Stability of metabolites in stock solutions, blood and plasma samples

Degradation of 100 $\mu g\,mL^{-1}$ 5-hydroxy- and 5,6-dihydroxythalidomide in methanol at −25°C was determined over a period of 48 days by direct injection into the chromatograph and comparison with freshly prepared solutions. Degradation of 100 $\mu g\,mL^{-1}$ 4,5-dihydroxy- and 5′-hydroxythalidomide in methanol was determined analogously over a period of 5 months and of EM 12, 100 $\mu g\,mL^{-1}$ in methanol, over a period of 21 days at 4°C. In addition, degradation of 4,5-dihydroxy- and 5′-hydroxythalidomide, 1 and 10 $\mu g\,mL^{-1}$ in 0·1 M phosphate buffer with 20 mM triethylamine (pH 3·5), was determined over a period of 80 days at 4°C.

The hydroxylated thalidomide metabolites were also added to freshly drawn heparinized human blood (concn 1·0 $\mu g\,mL^{-1}$). The mixtures were kept at 23°C in the dark. Duplicate samples for assay were taken after 0·5, 1·0, 1·5, 2, 3, 4, and 5 h. Twelve 0·5-mL samples containing 1·0 $\mu g\,mL^{-1}$ 5-hydroxy and 5,6-dihydroxythalidomide, and twelve containing 1·0 $\mu g\,mL^{-1}$ 4,5-dihydroxy- and 5′-hydroxythalidomide in fresh human plasma–buffer were also prepared. Six of each were assayed immediately; those remaining were assayed after 1·5–2 months at −25°C.

In-vitro incubations of liver homogenates

The incubations were performed according to a published method (Östraat et al 1996). Fraction S9 human liver homogenate, final protein concentration 1·0 or 4·0 $mg\,mL^{-1}$, and reaction buffer (0·13 M phosphate buffer, pH 7·4, 5 mM NADP, 6·5 mM glucose-6-phosphate, 10 mM $MgCl_2$ and 63 mM KCl) were added to sample tubes with *R*- or *S*-thalidomide at final concentrations of 200 $\mu g\,mL^{-1}$. The mixtures were incubated, with shaking, at 37°C in a water bath. Samples for determination of metabolites were taken after 0, 1, 2, 3 and 4 h, and stabilized as described above. Five separate experiments were performed with assays for 4,5-dihydroxy- and 5′-hydroxythalidomide and the samples were analysed on the day after incubation. Three experiments were performed with assays for 5-hydroxy- and 5,6-dihydroxythalidomide and the samples were analysed within two months. Some preliminary incubations were performed with 10% fraction S9 rabbit-liver homogenate (Östraat et al 1996) and racemic thalidomide at a final concentration of 50 $\mu g\,mL^{-1}$.

Plasma samples from man

Plasma samples for assay of metabolites were obtained during two different studies with human volunteers. The aim of the first study was to correlate the sedative effects of the thalidomide enantiomers with drug concentrations in blood (Höglund et al 1998); that of the second study was to compare rectal with oral administration of thalidomide. These results will be reported elsewhere. The study protocols were approved by the Ethics

Committee of the University of Lund and by the Swedish Medical Products Agency. Thus, samples for determination of 5-hydroxy- and 5,6-dihydroxythalidomide were taken from three healthy men who received 1·0 mg kg^{-1} (69–100 mg) *R*- or *S*-thalidomide on separate occasions as single oral doses. Samples for determination of 4,5-dihydroxy- and 5′-hydroxythalidomide were taken from six healthy men who had received 100 mg racemic thalidomide as a single oral dose. Blood samples (8–10 mL) were taken from an antecubital vein into heparinized tubes before administration of thalidomide and after 0·25, 0·5, 0·75, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 22 and 24 h. In addition, two male volunteers received 2·5 mg kg^{-1} (150 and 200 mg, respectively) of racemic thalidomide and samples were taken after 2, 3, 4, 5 and 6 h. The samples were immediately chilled in an ice-slush-filled cup and centrifuged at 4°C. The plasma was collected and acidified as described above. Blood samples (2–5 mL) for determination of *R*- and *S*-thalidomide were taken concomitantly. The samples were normally analysed within 4 days and within a maximum of 2 months.

Results

Representative chromatograms of the hydroxylated metabolites formed in S9 liver homogenates from man are given in Figures 1 and 2. Characteristics of the assays are shown in Table 2. The standard curves were linear ($r > 0.992$). The extraction yield for EM 12 was 78 ± 4·1%.

Comparison with freshly made solutions did not indicate any significant reduction in concentration when 5-hydroxy- or 5,6-dihydroxythalidomide was stored in methanol solution for 48 days at −25°C, when 4,5-dihydroxy- or 5′-hydroxythalidomide was stored for 5 months or when EM 12 was stored at 4°C for 21 days. When 4,5-dihydroxy- and 5′-hydroxythalidomide were stored in 0·1 M phosphate buffer with 20 mM triethylamine (pH 3·5) for 80 days at 4°C there was no degradation of 4,5-dihydroxythalidomide and 82% of 5′-hydroxythalidomide remained. After 5 h incubation in blood at room temperature 90% of the 5-hydroxy- and 82% of the 5,6-dihydroxythalidomide remained. The 4,5-dihydroxy and 5′-hydroxy metabolites were less stable, only 25 and 15%, respectively, remaining after 5 h. The corresponding half-lives of degradation were 2·9 and 2·2 h, respectively. No degradation of the 5-hydroxy or 5,6-dihydroxy metabolites was detected in acidified plasma stored at −25°C for 49 days. Of 4,5-dihydroxy- and 5′-hydroxythalidomide, 83 and 100%, respectively, remained after 2 months under these conditions.

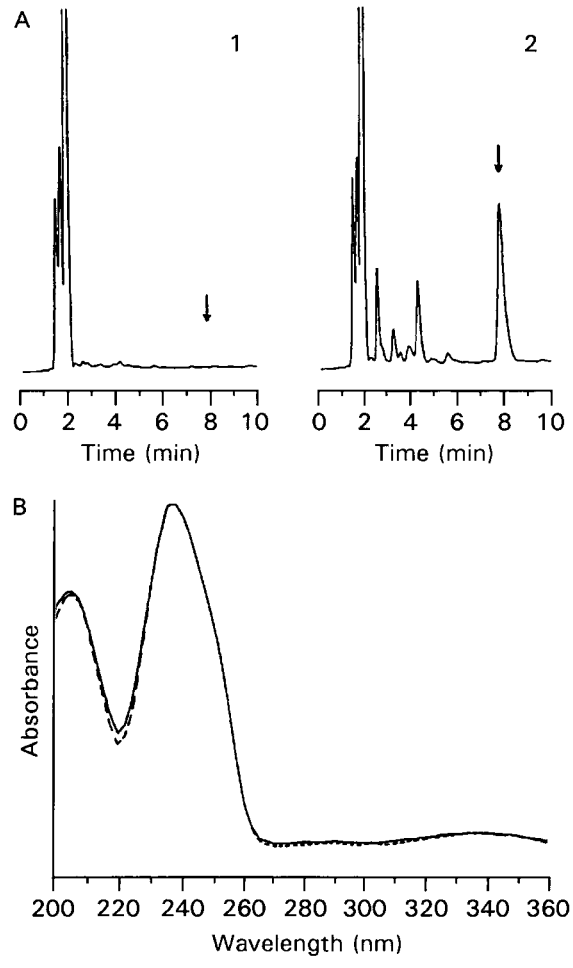


Figure 1. A. Chromatograms obtained from extracts of human S9 liver homogenates: 1, blank sample (0·6 mL); 2, sample (0·5 mL) containing 379 ng mL^{-1} 5-hydroxythalidomide formed during 4 h incubation of *R*-thalidomide (arrows indicate the retention time of the metabolite). For this chromatogram acetonitrile–buffer, 18 : 82, was used as mobile phase. The internal standard (phenacetin) eluted at 17·0 min. B. UV spectra recorded with the photodiode-array detector at the retention time of the 5-hydroxythalidomide peak. The reference spectrum (dashed curve) is from a blank homogenate to which 80 ng mL^{-1} synthetic metabolite had been added.

5-Hydroxy- and 5′-hydroxy- but not 4,5-dihydroxy- or 5,6-dihydroxythalidomide were detected after incubation of both enantiomers of thalidomide with human liver homogenate (Figures 1 and 2). The 5-hydroxy metabolite was formed at concentrations between 23 and 682 ng mL^{-1} during 2–4 h incubation. Its UV spectrum showed maxima at 205 and 238 nm (and a very flat maximum at 336 nm). The 5′-hydroxy metabolite was formed at concentrations of 8–89 ng mL^{-1} during 1–4 h incubation. Its UV spectrum showed one clear maximum at 222 nm, which was also the UV maximum of unchanged thalidomide. Incubations

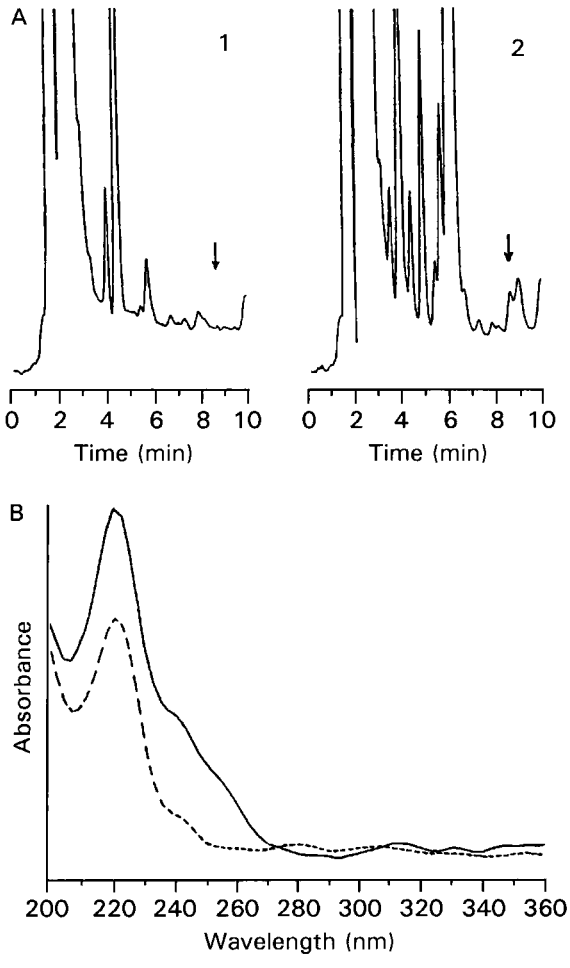


Figure 2. A. Chromatograms obtained from extracts of human S9 liver homogenates: 1, blank sample (0·6 mL); 2, sample (0·5 mL) containing 37 ng mL^{-1} 5′-hydroxythalidomide formed during 4 h incubation of *R*-thalidomide (arrows indicate the retention time of the metabolite). The internal standard (EM 12) eluted at 10·8 min. B. UV spectra recorded with the photodiode-array detector at the retention time of the 5′-hydroxythalidomide peak. The reference spectrum (dashed curve) is from a blank homogenate to which 10 ng mL^{-1} synthetic metabolite had been added. The shoulder at 250–265 nm in the spectrum of the metabolite is interference from the partially resolved later peak.

with either *R*- or *S*-thalidomide resulted in no clear differences between the formation of either metabolite. HPLC peaks with retention times corresponding to the 4,5-dihydroxy metabolite were also found. The photodiode array UV spectrum did not, however, correspond to that of the reference compound.

When racemic thalidomide was incubated with rabbit liver homogenate, 5-hydroxythalidomide was formed; its maximum concentration was 16 ng mL^{-1} after 5 h. 5,6-Dihydroxythalidomide could not be detected. Assays for the other two metabolites were not performed (reference compounds not being available). The half-life for degradation of thalidomide was 3–4 h.

The 5′-hydroxy metabolite was detected in all eight subjects after administration of racemic thalidomide (Figure 3) and the concentration–time profiles after sampling from the six subjects for 24 h are shown in Figure 4. HPLC with photodiode-array detection confirmed the identity of this metabolite, yielding a UV spectrum similar to the reference spectrum in Figure 2. Peaks with retention times corresponding to 4,5-dihydroxythalidomide were found in chromatograms of samples from five subjects. As for after in-vitro incubation, photodiode-array spectra showed that the peaks did not arise from this metabolite. The 5-hydroxy and 5,6-dihydroxy metabolites were not found; detection limits were 1 ng mL^{-1}.

Discussion

The main problem with the assay of thalidomide is that the compound is unstable in blood and plasma at physiological pH (Schumacher et al 1965a; Eriksson et al 1992, 1995, 1998; Eriksson & Björkman 1997); this is true also for the metabolites, in particular for 4,5-dihydroxy- and 5′-hydroxythalidomide. However, as for thalidomide itself (Eriksson et al 1992; Eriksson & Björkman 1997), acidification and freezing of the samples resulted in adequate storage stability, indicating that the absence of detectable quantities of some metabolites in the biological samples was not a result of ex-vivo degradation.

Metabolites of thalidomide hydroxylated in the aromatic ring have been tentatively identified in urine from man, rabbit and rat (Smith et al 1962). The metabolites were analysed by paper

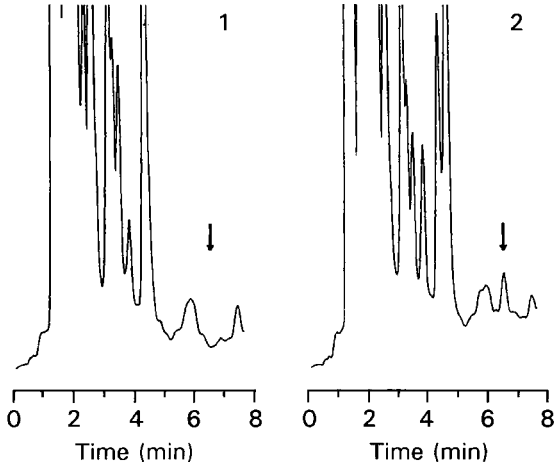


Figure 3. Chromatograms obtained from plasma samples from a healthy volunteer (photodiode-array detection). 1, sample taken before administration of thalidomide; 2, sample taken 6 h after an oral dose of 200 mg which contains 12 ng mL^{-1} 5′-hydroxythalidomide. The arrows mark the retention time of this metabolite.

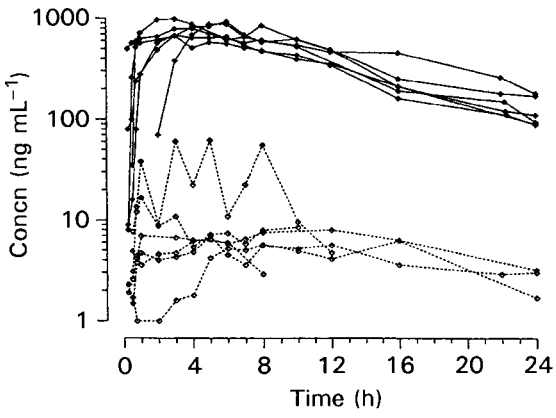


Figure 4. Concentration curves of thalidomide in blood (continuous) and of the 5′-hydroxy metabolite in plasma (dashed) in six human volunteers after oral administration of 100 mg thalidomide.

chromatography and one was believed to be a derivative of 3-hydroxyphthalic acid. Later, 4-hydroxy- and smaller amounts of 3-hydroxyphthalic acid were detected after acid hydrolysis of metabolites in rabbit urine (Schumacher et al 1965b). In-vitro 5-hydroxythalidomide was reported to be preferentially formed by *S*-thalidomide and 5′-hydroxythalidomide by *R*-thalidomide during incubation with rat liver homogenates (Knoche & Blaschke 1994; Weinz & Blaschke 1995). In laboratory animals thalidomide is eliminated mainly by spontaneous hydrolysis in blood, muscle and other tissues (Schumacher et al 1965b, 1968, 1970), but the rate of breakdown was considerably greater in the liver of monkeys and rabbits than in rat liver and other tissues (Schumacher et al 1968, 1970). This, however, was attributed to amidase activity and not to oxidative metabolism (Schumacher et al 1968).

We report the first identification of hydroxylated metabolites of thalidomide formed during incubation with liver homogenates from man. Our findings are at variance with a report that human microsomal preparations failed to catalyse the formation of hydroxylated thalidomide metabolites in-vitro (Zwingenberger & Wnendt 1996). The S9 fraction differs from microsomes in that it also contains a number of cytosolic enzymes. It seems unlikely, however, that metabolism by these enzymes could explain the discrepant findings. Instead, it is not clear from the previous report whether the investigators took adequate precautions against breakdown of the metabolites before the assay. We used several different batches of HepatoSNine and different concentrations of total liver protein in the incubations, which conceivably caused some of the variability in the yield of metabolites. Because the aim of this work was solely to investigate the possible formation of hydroxylated metabolites and identify them, the influence of these factors on the yield of metabolites was not studied systematically.

This is also the first identification of a hydroxylated thalidomide metabolite, albeit at low concentrations, in plasma from man. Plasma concentrations of the 5′-hydroxy metabolite from 100 mg racemic thalidomide were often close to the limit of detection of the assay. Higher doses were therefore given to two volunteers to facilitate identification of the metabolite by photodiode-array HPLC. Despite the technical difficulties identification of this metabolite is supported by its detection both in plasma and incubate samples. Also, the UV maximum of the metabolite was no different from that of thalidomide, as expected when hydroxylation has occurred in a position separate from the phthalimido chromophore.

The degradation of the 5′-hydroxy metabolite in blood at room temperature was more than five times faster than that of thalidomide itself, the half-life of which under these conditions was 11–13 h (Eriksson et al 1992). The metabolite is thus presumably more rapidly cleared in-vivo also, consistent with the low concentrations found in the circulation. The in-vivo half-life of the metabolite should be equal to that of the mother compound, because formation and not elimination would be the rate-limiting step in the pharmacokinetics of the metabolite. This was, however, difficult to ascertain because of the poor accuracy of the assay at the concentrations found in the plasma samples. The 5-hydroxy metabolite, despite being more stable in blood, was found only in-vitro and not in-vivo; this suggests it might be transformed further, e.g. by conjugation or hydrolysis, before reaching the circulation.

Because we could not synthesize the metabolites in our own laboratory they were procured for us by Grünenthal. Initially only 5-hydroxy- and 5,6-dihydroxythalidomide were available and the possible presence in plasma of these metabolites was investigated in samples from three participants in a study in which the separate enantiomers of thalidomide were given orally (and each subject thus contributed two series of plasma samples) (Höglund et al 1998). At this time also the preliminary incubations in rabbit liver homogenate were performed. Later 4,5-dihydroxy- and 5′-hydroxythalidomide became available and their possible presence was investigated in the plasma from eight subjects who were given racemic thalidomide. Because we have shown (Eriksson et al 1995) that orally administered enantiomers are extensively interconverted within a few hours in the

circulation, the pattern of metabolite formation should be qualitatively similar in the two studies.

Reported differences in species susceptibility to the teratogenic effects of thalidomide (Fratta et al 1965) might be because of differences in biotransformation, as observed in-vivo (Smith et al 1962; Schumacher et al 1965b, 1968, 1970) and in-vitro in liver homogenates (Schumacher et al 1968; Gordon et al 1981). *N*-hydroxythalidomide has been reported to be slightly more potent than thalidomide as a teratogenic agent in chicken embryos (Blaschke et al 1989). It has also been found that 3- and 4-hydroxythalidomide (in current nomenclature 4- and 5-hydroxy-) and some other derivatives hydroxylated in the 3- or 4-position of the phthalimido group can be teratogenic in this species (Boylen et al 1963). However, it should be noted that the typical foetal malformations seen in man can only be reproduced in primates and that reported differences between non-primate species in more general teratogenic effects of thalidomide must be interpreted with caution (Neubert & Neubert 1997). Gordon et al (1981) postulated that a toxic arene oxide metabolite of thalidomide might be involved in teratogenesis and demonstrated that liver preparations from rabbits or from foetuses from monkeys and man could produce (unidentified) metabolites of thalidomide that were toxic to human lymphocytes. Our finding that 5-hydroxythalidomide can be formed in the liver in man supports these earlier results and the putative formation of an arene oxide as a metabolic intermediate.

The effects on lymphocytes and some tumour cells of presumably formed hydroxylated or arene oxide metabolites have been described in several studies (Gordon et al 1981; Braun & Weinreb 1985; Hatfill et al 1991; Östraat et al 1996). In these studies thalidomide was incubated with rabbit liver homogenates for 1 or 2 h before being added to cell cultures to investigate whether thalidomide or the metabolites are active. Our pilot experiments confirmed that hydroxylated metabolites of thalidomide can be formed during incubation in rabbit S9 liver homogenate.

In summary, 5-hydroxy and 5′-hydroxy metabolites could be identified after in-vitro incubation of both enantiomers of thalidomide with liver homogenates from man. In circulating human blood 5′-hydroxy- but not 5-hydroxy-, 5,6-dihydroxy- or 4,5-dihydroxythalidomide could be identified with detection limits of 1–2 ng mL^{-1}.

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