Thalidomide Metabolites in Mice and Patients with Multiple Myeloma

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ABSTRACT

Purpose: This research examines the profile of metabolites of thalidomide that are formed in refractory multiple myeloma patients undergoing thalidomide therapy in comparison with those that are detected in healthy mice.

Experimental Design: Urine or plasma samples from patients during thalidomide therapy (100–400 mg daily), or from mice treated i.p. (100 mg/kg) or p.o. with thalidomide (50 mg/kg) were analyzed using liquid chromatography-mass spectrometry. Metabolites in each of the peaks observed in the UV- and mass spectrometry-detected high-performance liquid chromatography traces were identified by comparison of retention times and spectra with those of authentic standards.

Results: Plasma and urine samples from mice 4 h after treatment with thalidomide contained eight major metabolites formed by hydroxylation and/or hydrolysis of thalidomide. In contrast, urine samples from seven multiple myeloma patients at steady state levels of thalidomide therapy showed the presence of only three hydrolysis breakdown products and no hydroxylated metabolites.

Conclusions: Our results show that thalidomide metabolite profiles in multiple myeloma patients differ considerably from those in mice. The lack of measurable hydroxylated metabolites in urine and in 1 case plasma of these patients suggests that such metabolites are not responsible for the therapeutic effects of thalidomide in multiple myeloma. We suggest that thalidomide may act directly, down-regulating growth factors essential for multiple myeloma growth.

INTRODUCTION

Thalidomide (α-phthalimido glutarimide), marketed initially for its sedative effects (1) and later discovered to be effective in controlling acute symptoms associated with a number of inflammatory and autoimmune diseases (2, 3), has shown promise recently in the treatment of cancer (4). The clinical evaluation of thalidomide as an anticancer agent followed the demonstration that thalidomide could inhibit angiogenesis (5). Activity against renal carcinomas (6) and gliomas (7) has been observed, and thalidomide is also being trialled for the treatment of prostate cancers (8). The observation that patients with refractory multiple myeloma show an increase in bone marrow vascularization suggested that thalidomide might be used as an antiangiogenic agent (9). Although the resultant clinical trials with thalidomide have had outstanding results, no significant decrease in marrow microvessel density has been observed in patients responding to thalidomide therapy (10), suggesting that mechanisms other than antiangiogenesis may be involved in its clinical action.

Biotransformation of thalidomide occurs by hydrolysis (11–13) or by hepatic CYP-450-mediated hydroxylation (14), and both types of products are generally referred to as metabolites. Hydroxylated metabolites are present only in trace amounts after oral administration to humans but can be detected in vitro after incubation of thalidomide with human liver enzyme preparations (15, 16). Hepatic CYP2C19 activity is important for the 5- and 5'-hydroxylation of thalidomide in humans (15).

The teratogenic properties of thalidomide appear to require prior biotransformation (14, 17), and chirally stable hydrolysis products and arene intermediates have been implicated (18–20). On the other hand, the antiangiogenic effects and anticancer activities of thalidomide have been attributed to the formation of hydroxylated derivatives (21–23). The synthesis of hydroxylated thalidomide analogues has provided a number of compounds with potent antiangiogenic/antimetastatic activities (23), strengthening the hypothesis that hydroxylated thalidomide metabolites are responsible for antiangiogenic effects. The formation of thalidomide metabolites appears to be highly species-dependent and, whereas microsomal preparations from human, primates, or rabbits support the production of active metabolites, microsomes from rodents generally produce negative results (17, 19). These in vitro studies are consistent with the greater in vivo sensitivity to the effects of thalidomide observed in humans and rabbits as compared with that in rodents. However, inhibition of angiogenesis by thalidomide can be demonstrated in corneal assays with both rabbits and mice (5, 24, 25).

Because hydroxylated products of thalidomide have been proposed to be responsible for its antitumor effects, we have searched for the presence of these metabolites in multiple myeloma patients undergoing thalidomide therapy. We have developed methodologies where both hydrolysis and hydroxylated
metabolites of thalidomide can be detected in the one HPLC\(^3\) run using LC-MS, and have compared metabolite profiles in urine of mice and of patients with multiple myeloma. We have identified eight urinary metabolites in mice, including hydroxylated metabolites, but have not been able to detect these hydroxylated products in urine of patients treated with thalidomide. We have discussed the implications of these results to the mode of action of thalidomide in multiple myeloma.

**MATERIALS AND METHODS**

**Materials.** Racemic thalidomide for murine studies was synthesized at Celgene Corporation (Warren, NJ). OSA sodium salt was purchased from Riedel-de Haen (Seelze, Germany). Trichloroacetic acid, phthaloylglutamic acid, and β-glucuronidase were purchased from Sigma-Aldrich. CTAB and ACN were purchased from BDH Laboratory Supplies (Poole, United Kingdom), DMSO was purchased from Riedel-de Haen AG, and hydroxypropylcellulose was purchased from Glogau & Company Inc. (Melrose Park, IL).

**Authentic Standards.** Phthaloylglutamine and phthaloylisoglutamine were prepared by reaction of glutamine and isoglutamine, respectively, with N-carboethoxyphthalimide at room temperature in aqueous sodium carbonate solution. 4-Hydroxyphthaloylglutamine and 5-hydroxyphthaloylglutamine were synthesized by condensation of 3-hydroxyphthalic anhydride and 4-hydroxyphthalic anhydride, respectively, with glutamine in refluxing dry pyridine. Each was dehydrated using 1,1′-carbonyldimidazole and 4-(dimethylamino)pyridine in refluxing 

p-dioxane to give 4-hydroxythalidomide and 5-hydroxythalidomide. Similar reactions of 3- and 4-hydroxyphthalic anhydride with isoglutamine gave 4-hydroxyphthaloylisoglutamine and 5-hydroxyphthaloylisoglutamine, respectively. α-Aminoglutarimide was obtained as the hydrochloride salt starting from (N-carboxybenzoyl)glutamine as described else-

where (26). It was reacted with phthalic anhydride or 4-hydroxyphthalic anhydride in the presence of triethylamine, in dry tetrahydrofuran at room temperature to give N-(o-carboxybenzoyl)glutamic acid imide and 5-hydroxy-N-(o-carboxybenzoyl)glutamic acid imide, respectively. Similar reaction of phthalic anhydride with glutamine or isoglutamine at room temperature gave N-(o-carboxybenzoyl)glutamine and N-(o-carboxybenzoyl)isoglutamine, respectively. All of the synthetic standards were purified by chromatography on silica, and their structures were confirmed using 400 MHz 1H NMR spectroscopy and MS. 5'-Hydroxythalidomide was a generous gift from Prof. Sven Bjorkman (Malmo University Hospital, Malmo, Sweden) and was a mixture of 5'-cis and 5'-trans diastereomers.

**Mouse Studies.** Male 8–12-week-old C57/B1/6 mice bred at the Animal Resources Unit, Faculty of Medical and Health Sciences, University of Auckland were used for all of the experiments. Mice were housed under conditions of constant temperature and humidity according to institutional ethical guidelines. Thalidomide, dissolved in DMSO (40 mg/ml) was administered by i.p. injection (100 mg/kg, 2.5 μl/g body weight). For p.o. administration, thalidomide was suspended in 0.3% hydroxypropylcellulose (10 mg/ml) and administered using a gavage tube (50 mg/kg, 5 μl/g body weight). After drug administration, mice were placed in metabolic cages with water and food supplied, and urine over the 4-h period was collected. After 4 h, murine blood samples were collected in heparinized tubes during terminal halothane (NZ Pharmacology Ltd., Christchurch, New Zealand) anesthesia, centrifuged, and the plasma removed. Mouse plasma samples (300 μl each) and urine samples (100 μl each) were then acidified by adding 1N HCl up to 1 ml. Acidified samples were loaded onto 1 ml/100 mg preconditioned C18 Bond Elut columns (Varian, Harbor City, CA) using an automated sample preparation with an extraction column system (ASPEC; Gilson Medical, Middleton, WI). The columns were washed with 1 ml of Milli-Q water, and the compounds of interest were then eluted using 1 ml of 100% ACN. The eluates were evaporated to dryness using a centrifugal evaporator (Jouan, St. Nazaire, France) and residues reconstituted in 100-μl mobile phase for HPLC analysis.

**Human Studies.** Seven patients undergoing treatment with thalidomide for refractory multiple myeloma at Auckland Hospital were recruited for these studies. The thalidomide dosages of the patients ranged between 100 and 400 mg/day. Urine samples on 3 consecutive months of treatment as well as a plasma sample on one occasion was obtained from 1 patient with a >75% reduction in his IgG paraprotein on 100 mg thalidomide/day. Control samples of urine were obtained from healthy volunteers as well as a pretreatment sample from 1 of the patients. Urine (3.3 ml) or heparinized blood (0.8 ml) were acidified by adding 1 N HCl to 10 ml, and processed as described for murine samples. Dried residues of the samples were reconstituted in 500-μl mobile phase for urine samples and in 400-μl mobile phase for plasma samples.

**LC-MS Analysis.** Reconstituted samples were analyzed by LC-MS using an Agilent 1100 Series LC/MSD system (Agilent Technologies, Avondale, PA) using an atmospheric pressure chemical ionization interface. All of the samples were analyzed first using MSD set on negative-ion scan mode with a molecular weight range from 70 to 1000 a.m.u. to identify each of the peaks. Then, samples were reanalyzed on negative single-ion monitoring mode at the molecular weights 257, 273, 275, 276, 289, 291, 293, 294, and 449 a.m.u. (corresponding to each of the peaks) coupled with a second MSD signal set on negative-ion scan mode. This provided better sensitivity and reduced background noise. The other MS conditions were: fragmentor and capillary voltage of the interface, 100 and 3500 V, respectively; drying gas flow rate, 5 liter/min; corona current, 10 μA; gas temperature, 350°C; vaporizing temperature, 500°C; and nebulizing pressure, 35 p.s.i. Samples were also analyzed concurrently using diode array UV detection at 230 nm. The UV spectra of individual metabolite peaks were compared with those generated by the authentic standards (which had the same retention times), and the percentage match between the spectra determined using ChemStation Rev. A.08.04 (Agilent Technologies). Chromatographic separation was achieved with a LUNA 5 μ Phenylhexyl 100 × 4.6 mm stainless steel column (Phe-
nommenex, Torrance, CA) using a combination of the following solutions: solution A, which contained 80% ACN and 1% acetic acid in water, and solution B, which contained 10% ACN and 1% acetic acid in water. The elution program was 100% solution B at 0.5 ml/min over 0–20 min, addition of 0–20% solution A in a linear gradient at 0.7 ml/min over 20–45 min, and 100% solution B at 0.5 ml/min over 45–55 min.

Resolution of Phthaloylglutamine and Phthaloylisoglutamine by HPLC. The chromatographic conditions used in the LC-MS analyses did not allow the separation of phthaloylglutamine and phthaloylisoglutamine. The mobile phase was modified by the addition of the ion pair reagents CTAB and OSA. Analysis of these two compounds was performed using a Waters semi-preparative HPLC system (Waters Associates, Milford, MA) consisting of a 717PLUS auto sampler, 1525 binary HPLC pump, 100 × 10.0 mm stainless steel LUNA 5µ Phenylhexyl column (Phomenex) and a model 2487 dual λ absorbance detector set at 230 nm, and the peak containing a mixture of phthaloylisoglutamine and phthaloylglutamine was collected with a Gilson model-202 fraction collector (Gilson Medical Electronics, Middleton, WI). The mobile phase consisted of 10% ACN and 1% acetic acid in Milli-Q water, and the flow rate was 0.5 ml/min isocratic. The collected eluant was dried using a Virtis Freeze-Mobile 6 model freeze dryer (Virtis Co., Inc., Gardiner, NY). The reconstituted dried samples were analyzed by the same Waters HPLC system but using a LUNA 5µ Phenylhexyl 100 × 4.6 mm stainless steel column instead of 100 × 10.0 mm stainless steel LUNA 5µ Phenylhexyl column, and a mobile phase of 10% ACN and 1% acetic acid in Milli-Q water, 48 mg/liter OSA, and 32 mg/liter CTAB. Peaks were monitored by UV at 230 nm using an isocratic flow rate of 0.5 ml/min.

Thalidomide Glucuronide Identification. The fraction containing the glucuronide metabolite of thalidomide was collected and dried using the same procedure as that described above for the phthaloylisoglutamine/phthaloylglutamine fraction. The method of identification was adapted from that of Webster et al. (27) used to identify glucuronidation of 5,6-dimethylxanthenone-4-acetic acid. In brief, the dried residue was reconstituted in 0.1 M PBS (pH 5.5), and two aliquots of 500-µl reconstituted metabolite solution were incubated at 37°C for 45 min and 90 min with 2000 units/ml β-glucuronidase plus 20 mM D-saccharic acid 1,4-lactone, respectively. Another two aliquots of 500-µl reconstituted metabolite solution were incubated together without β-glucuronidase as control. The reaction was initiated by addition of β-glucuronidase and D-saccharic acid 1,4-lactone, and stopped by addition of 50 µl of 10% trichloroacetic acid. The mixture was centrifuged at 3000 × g for 15 min to remove precipitated protein. The supernatant was removed then injected into LC-MS, and analyzed using the same procedure used to analyze mouse and human plasma and urine samples.

RESULTS

Detection of Thalidomide Metabolites in Mice. Mice were treated with thalidomide either i.p. (100 mg/kg) or p.o. (50 mg/kg), and urine and plasma samples were collected at 4 h and analyzed using LC-MS. The UV profiles of urine and plasma from both untreated and treated mice showed a number of peaks (Fig. 1A), and differences in the UV profiles were used to detect metabolites. Peaks were considered to be metabolite peaks if they gave signals on MS-negative ion scan mode and negative single-ion monitoring mode (Fig. 2A and Fig. 3A), and if the mass spectrum of the peak contained additional ions to those in untreated control samples. An example for peak 6, is shown for scan mode in Figs. 2A and 3A and in Fig. 3, A and B. By these criteria, seven metabolite peaks, in addition to the thalidomide peak, were identified in the urine from mice treated with thalidomide (Fig. 1A).

Identification of Biotransformation Products. Possible metabolites with molecular weights corresponding to the MS signals of each of the peaks were deduced, and authentic samples of the majority of these were obtained. The retention times, UV spectra, mass spectra, and single ion monitoring chromatographic profiles of metabolite peaks and authentic standards were compared. Results are summarized in Table 1. Peak 1, with a molecular mass of 276, was identified as N-(o-carboxybenzoyl)glutamic acid imide on the basis that they had the same retention time, and their UV spectra shared 93.5% identity to each other (Fig. 4A). Peak 2, with a mass of 292, could correspond to a variety of possible hydroxylated hydrolysis products. Therefore, we synthesized 5- and 4-hydroxycarboxybenzoylglutamine, 5- and 4-hydroxyphthaloylglutamine, and 5- and 4-
hydroxy-N-(o-carboxybenzoyl)glutamic acid imide, and found they all had different retention times to peak 2. N-o-hydroxy-N-(o-carboxybenzoyl)glutamic acid imide also has a mass of 292, but metabolites resulting from N-hydroxylation of the imide ring of thalidomide have never been detected (15, 16, 28, 29), and it is unlikely that this is the compound in peak 2. We suggest that the most likely candidate for peak 2 is N-o-hydroxy-N-(o-carboxybenzoyl)glutamic acid imide, although the authentic compound is not yet available for confirmation.

Peak 3, a major component of the urine extracts, showed two molecular ions, corresponding to components with molecular weights of 450 and 274. It eluted just before an unidentified host component (present in urine of untreated mice) that had a molecular weight of 179. The molecular weight of the larger component suggested that it was an O-glucuronide derivative of thalidomide, whereas that of the smaller component suggested that it was a fragment ion resulting from loss of glucuronic acid. Therefore, peak 3 was collected, a portion was treated with α-glucuronidase, and both portions were rechromatographed (Fig. 5A). Exposure to α-glucuronidase caused the appearance of a new component, peak II, with a mass spectrum corresponding to a molecular weight of 274 (Fig. 5B). This was identified as 5-hydroxythalidomide on the basis that it has the same retention time, and its UV spectrum shares 99% identity with that of authentic material. Peak I had a molecular weight of 450 (its reduced retention time was because of the lower loading of the column), and the decrease in its proportion after exposure to β-glucuronidase strongly suggested that it was thalidomide-5-O-glucuronide. The peak eluting at 12.8 min (molecular weight 179) corresponded to the unidentified host component. No evidence of glucuronidation at the 5'-position was found.

Peak 4 had a molecular mass of 276, and identical UV and mass spectra to authentic phthaloylisoglutamine. However, authentic phthaloylglutamine also has the same mass and has a very similar retention time, and it was necessary to determine whether both might be present in peak 4. Therefore, this fraction was collected and reanalyzed by HPLC using the mobile phase containing CTAB and OSA that allows separation of phthaloylisoglutamine and phthaloylglutamine with respective retention times of 15.1 min and 12.7 min (Fig. 6A). The sample collected from peak 4 resolved into two fractions (Fig. 6B), indicating that both phthaloylglutamine and phthaloylisoglutamine were present.

Peaks 5, 6, and 7 were identified as cis-5'-hydroxythalidomide, trans-5'-hydroxythalidomide, and 5-hydroxythalidomide, respectively, on the basis of similarity of UV spectra (Fig. 4, B–D) and identity of retention time (Table 1). The same number of metabolite peaks in UV profiles was obtained in urine after p.o. or i.p. administration (Fig. 7). The profile obtained in plasma contained smaller-sized but the same number of peaks compared with the urine profile from mice administered thalidomide i.p. at the same dose (Fig. 8A).

**Thalidomide Metabolites in Multiple Myeloma Patients.** Seven patients with refractory multiple myeloma, receiving between 100 and 400 mg thalidomide daily, were recruited for this study. Urine samples from patients on thalidomide therapy were analyzed for metabolites using the same procedure as that for murine samples. A pretreatment
sample from one patient (Fig. 1B) showed no significant difference to those from healthy individuals, and peaks observed in untreated controls were not included in the analysis. Applying the same criteria as those used for defining murine metabolite peaks, only two metabolite peaks were detected in the UV profile of all seven of the patients’ urine (Fig. 1B; Fig. 8B). The first of these peaks was the same as peak 1 in the murine profile, corresponding to N-(o-carboxybenzoyl)glutamic acid imide, whereas the second was identical to peak 4. This second peak was reanalyzed by HPLC using the ion-paired mobile phase, and like the corresponding peak in mice, it could be resolved into two components corresponding to phthaloylglutamine and phthaloylisoglutamine (Fig. 6C).

Patient 1, who showed a >75% reduction in paraprotein

**Table 1** Metabolite peaks in UV profiles from murine urine after thalidomide treatment

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Retention time (min)</th>
<th>Molecular weight</th>
<th>Match of UV spectra</th>
<th>Metabolite</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.4</td>
<td>276</td>
<td>93.5%</td>
<td>N-(o-carboxybenzoyl)glutamic acid imide</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.1</td>
<td>292</td>
<td></td>
<td>5’-hydroxy-N-(o-carboxybenzoyl)glutamic acid imide&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12.8</td>
<td>450</td>
<td></td>
<td>thalidomide-5-O-glucuronide</td>
<td></td>
</tr>
<tr>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.5</td>
<td>276</td>
<td></td>
<td>phthaloylglutamine</td>
<td></td>
</tr>
<tr>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.5</td>
<td>276</td>
<td></td>
<td>phthaloylisoglutamine</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25.4</td>
<td>274</td>
<td>98%</td>
<td>cis-5’-hydroxy-thalidomide</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>31.3</td>
<td>274</td>
<td>98%</td>
<td>trans-5’-hydroxy-thalidomide</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>33.3</td>
<td>274</td>
<td>&gt;99%</td>
<td>5-hydroxythalidomide</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Chiral centre.
<sup>b</sup> See Fig. 4.
<sup>c</sup> Proposed metabolite.
<sup>d</sup> Peak 4 separates into 4a and 4b with retention times of 12.7 and 15.1 min, respectively, on HPLC with mobile phase containing CTAB and OSA.
level on thalidomide, was studied while on a dose of 100 mg/day. Urine samples collected on 3 consecutive months of treatment were analyzed, but no variations in the metabolite profiles were seen over this period. This patient also provided a blood sample 15 h after 1 of his daily thalidomide doses. Thalidomide metabolites were not detected in this plasma although they were detectable in a urine sample collected at the same time (Fig. 8B).

**DISCUSSION**

The results confirm the formation in mice of three first-step hydrolysis products, N-(o-carboxybenzoyl)glutamic acid imide, phthaloylisoglutamine, and phthaloylglutamine, as well as three first-step hydroxylation products, 5-hydroxythalidomide, and cis- and trans-5'-hydroxythalidomide. In addition, we provide evidence of a second-step transformation of 5-hydroxythalidomide to produce thalidomide-5'-O-glucuronide. A scheme for these transformation steps is shown in Fig. 9. Although several studies report the presence of unidentified metabolite peaks (15, 16, 30), glucuronidated thalidomide metabolites have not been documented previously. Glucuronidation confers greater solubility to a compound, and the formation of glucuronidated metabolites may facilitate faster metabolism and excretion via this route. Indeed, the thalidomide-5-O-glucuronide was the largest metabolite peak in mouse urine. Glucuronidation occurred only at the 5-position, and no evidence for the formation of thalidomide-5'-O-glucuronide was found.

Although we have not been able to validate the structure by comparison with authentic material, we suggest that the metabolite in peak 2 is 5'-hydroxy-N-(o-carboxybenzoyl)glutamic acid imide, which can be formed either by second-step hydrolysis of 5'-hydroxythalidomide or second step hydroxylation of N-(o-carboxybenzoyl)glutamic acid imide. Other authentic standards with the same mass have been ruled out, because they have different HPLC retention times.

We found striking differences in the urinary thalidomide metabolite profiles between mice and patients with multiple myeloma. In the murine profiles, seven peaks containing eight metabolites formed by hydrolysis or hydroxylation of 5'-hydroxythalidomide or second step hydroxylation of N-(o-carboxybenzoyl)glutamic acid imide. Other authentic standards with the same mass have been ruled out, because they have different HPLC retention times.

We found striking differences in the urinary thalidomide metabolite profiles between mice and patients with multiple myeloma. In the murine profiles, seven peaks containing eight metabolites formed by hydrolysis or hydroxylation (Fig. 1A; Fig. 8A; Table 1) were observed, whereas in the profiles of patients with multiple myeloma, only three products were detectable, all produced by hydrolysis (Table 2; Fig. 1B; Fig. 8B). Because p.o. and i.p. administration of thalidomide to mice produced essentially identical metabolite profiles (Fig. 7), the differences are unlikely to be because of an altered route of administration and may, therefore, reflect differences in metabolism. It has been found that the quantities of 5- and 5'-hydroxy...
thalidomide metabolites, thought to be produced by CYP2C19 activity in human liver microsomes, are \textasciitilde 10-fold lower than those obtained with liver microsomes from rodents, where CYP2C6 is the primary enzyme responsible (15). Thus, thalidomide appears a much poorer substrate for the human CYP enzymes involved compared with the equivalent rodent enzymes, explaining the lower level of hydroxylation of thalidomide in humans.

Because plasma samples were only analyzed in one patient (Fig. 8B), we cannot exclude the possibility that hydroxylated thalidomide metabolites are produced in plasma of humans but do not appear in urine. However, in patients with Hansen’s disease given a single p.o. dose of 400 mg thalidomide, no metabolites were detected in plasma, and the 5-hydroxy metabolite, although detected in urine, was below the limits of quantitation (29). Furthermore, in a study using healthy male volunteers, the plasma concentration of 5'-hydroxylated metabolite was of the order of 0.1% of that of thalidomide, although both 5'- and 5-hydroxy metabolites were formed \textit{in vitro} in the presence of human S9 liver fractions (16). In addition to the intrinsically lower amounts of hydroxylation in humans, it is possible that this pathway is suppressed in multiple myeloma patients as a result of their disease. A study involving 16 patients with advanced cancer showed a reduction in metabolic activity, correlating with decreased CYP2C19 activity, as compared with healthy volunteers (31).

The lack of detectable hydroxylated metabolites in multiple myeloma patients who are responding to thalidomide therapy suggests that such metabolites are not responsible for the therapeutic effect. Because hydroxylated metabolites have been implicated in the inhibition of angiogenesis, the results also
imply that the primary mechanism of action of thalidomide in responding multiple myeloma patients does not involve antian-
giogenesis. An alternative explanation is that thalidomide itself is the active agent and exerts its activity by immunomodulation. The progression of multiple myeloma is strongly dependent on interleukin-6 and vascular endothelial growth factor production triggered by adhesion of the tumor cells to bone marrow stromal cells (32, 33). The ability of thalidomide to inhibit the biosyn-
thesis of a range of cytokines, including interleukin-6, is well
documented (34–36), and inhibition of growth factors necessary for tumor cell survival provides an alternative mechanism of action of thalidomide in the therapy of multiple myeloma. Inhibition of cytokine biosynthesis by thalidomide is not de-
pendent on prior hepatic activation and occurs efficiently in vitro (37). Rather, this activity is dependent on the intact parent compound and is lost on hydrolysis (38). We suggest that down-regulation of essential growth factors is the basis of the therapeutic effects of thalido-
mide in multiple myeloma.

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