Review

Clinical Evidence of Pharmacokinetic Changes in Thalidomide Therapy

Katsunori Nakamura1, Naoki Matsuzawa2, Shigeru Ohmori2, Yuichi Ando3, Hiroshi Yamazaki4 and Tamihide Matsunaga1, *

1Department of Clinical Pharmacy, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan
2Department of Pharmacy, Shinshu University Hospital, Matsumoto, Japan
3Department of Clinical Oncology and Chemotherapy, Nagoya University Hospital, Nagoya, Japan
4Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Machida, Japan

Summary: The teratogenic effects of thalidomide have been studied for more than 50 years. However, there have been few studies of the pharmacokinetic changes occurring during thalidomide therapy. Thalidomide was originally developed as a sedative. However, thalidomide induces multiple birth defects when used in pregnant women. Thalidomide is now used in the treatment of multiple myeloma (MM) and erythema nodosum leprosum (ENL) in Japan. Rational use of thalidomide is problematic due to a lack of basic research regarding its mechanism of action and serum concentration/effect relationships. There are a number of hypotheses for pharmacokinetic changes in thalidomide therapy. Genetic factors including single nucleotide polymorphisms (SNPs) that change cytochrome P450 (CYP) activity and epigenetic regulation that modifies CYP expression levels may contribute to the changes in pharmacokinetics and adverse drug reactions (ADRs) of thalidomide. Environmental factors include the pharmacological context of drug-drug interactions and the physiological context of liver diseases. Liver and kidney diseases do not play important roles in pharmacokinetic changes or ADRs in thalidomide therapy. To date, most research has focused on teratogenic activity, while the impact of polymorphisms in genes encoding drug metabolic enzymes and drug-drug interactions could mediate ADRs. Here, we discuss clinical evidence of pharmacokinetic changes in thalidomide therapy.

Keywords: thalidomide; CYP2C19; CYP3A5; polymorphism; inter-individual variability

Introduction

Thalidomide was once used as a nonbarbiturate sedative-hypnotic with only low toxicity, but was withdrawn from the market because of its teratogenic side effects in the early 1960s.1 Cereblon, a protein encoded by a candidate gene for mild mental retardation, was shown to be a primary target of thalidomide teratogenicity due to the inhibition of ubiquitin ligase activity.2 Thalidomide has attracted renewed interest in recent years for use on a restricted basis in certain countries as a novel antineoplastic agent with immunomodulatory and antiangiogenic activities.3–6 This drug is now commonly used in the treatment of multiple myeloma (MM).7–9 Thalidomide has also been shown to have some activity against renal cell carcinoma, Kaposi’s sarcoma, agnogenic myeloid metaplasia, Waldenström’s macroglobulinemia, and myelodysplastic syndrome.10,11 However, limited efficacy data are available in these conditions compared to myeloma, and its use in these settings is still investigational. Studies of its use in several other malignant and nonmalignant disorders are currently ongoing.12 The incidence of toxicity is correlated with the dose of the drug. Patients receiving 200 mg or less seem to tolerate the treatment well with minimal side effects. Conversely, almost all patients taking thalidomide at doses of more than 400 mg/day experience drug-related toxicity.12 Thalidomide is eliminated mainly through nonenzymatic hydrolysis and urinary excretion in humans, although animal studies have suggested that it may also be metabolized by the hepatic CYP enzyme system.13–16 (S)-Thalidomide was reported to induce apoptosis.17 However, (S)-thalidomide was reported to undergo rapid conversion into the racemate in vitro.18,19 The transition of (R)- and (S)-thalidomide could influence the therapeutic effects. The increasing use of thalidomide raises the possibility of metabolic interactions with other prescription medications.20 Clinically important interactions between thalidomide and coadministered therapeutic agents may
affect the activation or detoxification of thalidomide and other drugs, giving rise to attenuation or amplification of biological effects and/or toxicities. While metabolic studies have been performed in animals to date there have been only a few human studies. This review summarizes the clinical evidence of pharmacokinetic changes in thalidomide therapy.

**Metabolism**

**Metabolism of thalidomide in humans:** Thalidomide is an optical isomer, and (S)-thalidomide was reported to induce apoptosis. However, rapid conversion occurs from (S)-thalidomide into the racemate in vitro. It has been suggested that the transition of (R)- and (S)-thalidomide in the blood influences the therapeutic effects. Although thalidomide is mainly eliminated through nonenzymatic hydrolysis and urinary excretion in humans, animal studies have suggested that it may also be metabolized by the hepatic CYPs. Thalidomide was oxidized to 5-hydroxythalidomide and 5’-hydroxythalidomide by NADPH-fortified liver microsomes from humans as observed in monkeys, rats, mice, rabbits, and dogs (Fig. 1). (R)-Thalidomide was hydroxylated more efficiently than (S)-thalidomide. Thalidomide has been reported to be metabolized by human recombinant CYP2C19, 3A4, and 3A5 in vitro. CYP3A is known to be the main enzyme involved in the metabolism of many currently available medications. It is commonly accepted that CYP3A5 substrate specificity is similar to that of CYP3A4, although some differences in catalytic properties of thalidomide metabolism have been found. Human CYP2C19, 3A4, and 3A5 mediate thalidomide 5-hydroxylation and further oxidation leading to a glutathione conjugate, which may be of relevance in the pharmacological actions of thalidomide (Fig. 1). These metabolic pathways were confirmed by humanized TK-NOG mice, prepared by the introduction of thymidine kinase followed by induction with ganciclovir, and transplantation of human liver cells. Experiments in these humanized mice could be expected to reveal the pharmacokinetic differences between humans and other animals. As thalidomide metabolites were not stable, degradation was avoided by rapid chilling and acidification of the samples. This property makes it more difficult to perform kinetic analysis of thalidomide. After incubation of thalidomide with the S9 fraction from human liver, formation of the 5-hydroxy and 5’-hydroxy metabolites was observed. The 5’-hydroxy metabolite was found in plasma samples from eight healthy male volunteers who had received thalidomide orally, but the concentrations were low. Thalidomide does not undergo significant metabolism by human CYP and clinically important interactions between thalidomide and drugs that are also metabolized by this enzyme system are unlikely (Table 1). The major route of thalidomide breakdown in humans and animals is through spontaneous hydrolysis with subsequent elimination in the urine. As both enzymatic metabolism and renal excretion play minor roles in the elimination of thalidomide, the risk of drug interactions seems to be low.

**CYPs and Transporters**

**Genetic polymorphisms and thalidomide pharmacokinetic changes:** CYP is one of the most important groups of enzymes

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**Table 1. Summary of thalidomide-related pharmacokinetic changes from drug-drug interactions**

<table>
<thead>
<tr>
<th>Substrate and inhibitor</th>
<th>Effect</th>
<th>Mechanism of pharmacokinetic change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs affecting the pharmacokinetics of thalidomide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>inhibition</td>
<td>Cyclophosphamide increased the thalidomide t(1/2) and AUC in plasma and tumor tissue in mice</td>
</tr>
<tr>
<td>CPT-11</td>
<td>no change</td>
<td>CPT-11 did not significantly alter the pharmacokinetics of thalidomide</td>
</tr>
<tr>
<td></td>
<td>no change</td>
<td>Concurrent administration of CPT-11/thalidomide did not influence pharmacokinetics</td>
</tr>
<tr>
<td>Thalidomide affecting the pharmacokinetics of other drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>enhancement</td>
<td>Cyclosporin metabolism was enhanced by thalidomide in CYP3A5 and in liver microsomes expressing CYP3A5 in the presence of thalidomide</td>
</tr>
<tr>
<td></td>
<td>enhancement</td>
<td>Cyclosporin A clearance was enhanced in CYP3A5 and liver microsomes expressing CYP3A5 by thalidomide</td>
</tr>
<tr>
<td>CPT-11 and SN-38</td>
<td>no change</td>
<td>Thalidomide inhibited CPT-11 metabolism, unlikely to be clinically significant</td>
</tr>
<tr>
<td></td>
<td>inhibition</td>
<td>Thalidomide increased the AUC of CPT-11</td>
</tr>
<tr>
<td></td>
<td>inhibition</td>
<td>Thalidomide decreased the AUC and t(1/2) of SN-38</td>
</tr>
<tr>
<td></td>
<td>no change</td>
<td>Thalidomide decreased metabolism of CPT-11 into SN-38</td>
</tr>
<tr>
<td>Midazolam</td>
<td>inhibition</td>
<td>Midazolam 4-hydroxylation activities were suppressed by thalidomide</td>
</tr>
<tr>
<td></td>
<td>enhancement</td>
<td>1’-Hydroxylation and total midazolam oxidation were enhanced in the presence of thalidomide</td>
</tr>
<tr>
<td></td>
<td>enhancement</td>
<td>Midazolam hydroxylation was enhanced by thalidomide in CYP3A5 and in liver microsomes expressing CYP3A5 in the presence of thalidomide</td>
</tr>
<tr>
<td>(S)-Mephenytoin</td>
<td>inhibition</td>
<td>Thalidomide inhibited (S)-mephenytoin 4'-hydroxylation activities of recombinant CYP2C19 and human liver microsomes</td>
</tr>
<tr>
<td>Testosterone</td>
<td>enhancement</td>
<td>Testosterone 6β-hydroxylation were enhanced in the presence of thalidomide</td>
</tr>
<tr>
<td>DMXAA</td>
<td>inhibition</td>
<td>Thalidomide reduced clearance of DMXAA</td>
</tr>
<tr>
<td></td>
<td>no change</td>
<td>(S)-Thalidomide did not alter plasma DMXAA AUC in rats</td>
</tr>
</tbody>
</table>

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in thalidomide metabolism. Many CYPs are polymorphic and catalytic alterations of allelic variant proteins can affect the metabolic activities of many drugs. The CYP2C19, 3A4, and 3A5 genes, the gene products of which catalyze thalidomide hydroxylation, are particularly polymorphic. In vitro studies using cDNA expression systems are useful tools for evaluating functional alterations of the allelic variants of CYP, particularly for low-frequency alleles. There was some influence of genetic polymorphism in CYP2C19 on the blood concentration of thalidomide in MM, amyloid light chain amyloidosis, and polynuropathy, organomegaly, endocrinopathy, monoclonal gammapathy, and skin changes (POEMS) syndrome related to MM in Japanese patients.

It has been reported that decreased formation of thalidomide metabolites would be expected with defective alleles of CYP2C19 compared to wild-type in clinical treatment with thalidomide plus dexamethasone. Association studies of genetic variation and treatment effect may serve as predictive markers for the effects of treatment and can also uncover biological pathways behind drug effects. The SNPs have been studied in relation to high-dose treatment, thalidomide- and bortezomib-based therapy, maintenance treatment with interferon-α, and therapy-related adverse effects caused by treatment. In thalidomide- and bortezomib-based therapy, candidate genes include tumor necrosis factor-α (TNF-α) and genes involved in the nuclear factor kappa B pathway, respectively. In maintenance treatment with interferon-α, a polymorphism in gene NFkB1 is a candidate for prediction of efficacy. Adverse drug reactions (ADRs) include infection, osteonecrosis of the jaw, venous thrombotic events (VTE), and peripheral neuropathy (PN). A SNP in the CYP2C8 gene was strongly associated with osteonecrosis of the jaw. Several SNPs in genes encoding proteins involved in DNA repair, apoptosis, and inflammation as well as genes involved in nervous system function are associated with VTE induced by thalidomide and PN induced by bortezomib.

Further studies of SNPs in clinical trials are needed. Relation of transporters to the pharmacokinetic changes of thalidomide: Since studies in patients have indicated that the oral absorption of thalidomide is considerably variable at high doses, the contributions of transporters to the pharmacokinetics/pharmacodynamics of thalidomide were examined by Zhou et al. and Zimmermann et al. using human colon cancer cell lines, which have been widely used to investigate drug permeability. Thalidomide did not induce P-glycoprotein (P-gp) expression in LS180 cells. The uptake of rhodamine 123 in CCRF cells overexpressing P-gp was not influenced by coinubcation with thalidomide. Transport through Caco-2 monolayers was linear and the permeability was similar in both directions. There were no differences between the thalidomide enantiomers. From this study, thalidomide was concluded to be neither a substrate nor an inhibitor or inducer of P-gp, and P-gp-related drug-drug interactions with thalidomide are unlikely. Zhou et al. reported that the uptake of thalidomide by Caco-2 cells was very limited (up to 2.1%). The transport of thalidomide appeared to be linear up to 1 h. The permeability coefficients (Papp) of thalidomide at 2.5–300 μM from apical (AP) to basolateral (BL) and from BL to AP were 2 × 10^{-5}–6 × 10^{-5} cm/s, with a marked decrease in Papp values from AP to BL at increased thalidomide concentration. The transport of thalidomide was dependent on sodium, temperature, and pH, as replacement of extracellular sodium chloride or reducing temperature and apical pH resulted in significant decreases in the Papp values. Additional data indicated that transport of thalidomide is energy-dependent, as it was significantly (p < 0.05) inhibited by the ATP inhibitors sodium azide and 2,4-dinitrophenol. In addition, α-glutamic acid, cystidine, dipyridamole, papaverine, quinidine, and cyclophosphamide significantly (p < 0.05) inhibited the transport of thalidomide, while the P-gp inhibitor verapamil and other nucleosides and nucleotides, such as thymidine and guanine, had no effect. These results suggested that thalidomide may be rapidly transported by a saturable energy-dependent transporter in Caco-2 monolayers.

Drug-drug interaction of thalidomide: The increasing use of thalidomide raises the possibility of metabolic interactions with other prescription medications. Clinically important interactions between thalidomide and coadministered therapeutics may affect the activation or detoxification of thalidomide and other drugs giving rise to attenuation or amplification of biological effects and/or toxicities. While metabolic studies have been performed in animals, to date there have been only a few human studies (Table 1). The possible drug interactions that could be mediated by thalidomide were investigated in human liver microsomes. (S)-Mephentoyin 4-hydroxylation activity was inhibited by thalidomide in recombinant CYP2C19 and human liver microsomes with apparent IC50 of approximately 270 μM. Interestingly, midazolam 4-hydroxylation activity was suppressed by the presence of thalidomide, but 1’-hydroxylation activities, total midazolam oxidation activity, and testosterone 6β-hydroxylation activities were enhanced in the presence of thalidomide. Recombinant CYP3A4 altered kinetics at clinical concentrations of thalidomide. CYP3A4 was affected only at higher thalidomide concentrations. Enhancement of midazolam hydroxylation by thalidomide was seen in liver microsomes from CYP3A4*1 subjects. Cyclosporin A clearance was similarly enhanced by thalidomide in recombinant CYP3A4 and liver microsomes expressing CYP3A4. Close interaction between thalidomide and the heme of CYP3A4 was observed in docking studies. As total midazolam metabolism or cyclosporin A clearance may be increased by thalidomide in a dose-dependent manner, unexpected drug interactions could occur via heterotropic cooperativity of CYP3A4. Both (S)-thalidomide and diclofenac increased the plasma DMXAA AUC in mice. In the case of diclofenac, this may be due to direct competitive inhibition of DMXAA metabolism, but this mechanism is not always applied to (S)-thalidomide. The in vivo predictive model is inappropriate for the (S)-thalidomide DMXAA interactions when based on direct inhibition of metabolism in mice and humans. Thalidomide tends not to affect the pharmacokinetics of orally administered hormonal contraceptives. In contrast, conversion of CPT-11 into the active metabolite SN-38 was significantly inhibited by thalidomide. The possibility of an interaction of thalidomide with CPT-11 metabolism may explain the previously described improvement in tolerability of CPT-11 therapies.
in patients taking CYP2C19 substrates. CYP2C19 genetic polymorphism may be one of the factors underlying the individual differences in thalidomide toxicity. The mean (R)-thalidomide AUC was 35.9% lower in the CYP2C19*1/*3 and *1/*2 groups than in the CYP2C9*2/*2 group, and the mean (S)-thalidomide AUC was 33.5% lower in the CYP2C19*1/*3 and *1/*2 groups than the CYP2C9*2/*2 group. All patients developed adverse reactions to thalidomide. Major adverse drug effects were constipation, somnolence, and peripheral neuropathy. Only one patient with the CYP2C19*2/*2 genotype taking thalidomide developed dyspnea as a side effect, but it improved following the termination of thalidomide. Peripheral neuropathy is a common side effect of thalidomide and often calls for the cessation of therapy when the symptoms are severe. The thalidomide-related peripheral neuropathy associated with ABCA1 (rs363717), ICAM1 (rs1799969), PPARD (rs2076169), SERPINB2 (rs6103), and SLC12A6 (rs7164902) SNPs and an individual's risk of developing peripheral neuropathy after thalidomide treatment can be mediated by polymorphisms in genes governing repair mechanisms and inflammation in the peripheral nervous system. No association was observed between the number of functional CYP2C19 and CYP2D6 alleles and outcome in a population of 166 MM patients treated with thalidomide. There were also no associations between the numbers of functional CYP2C19 and CYP2D6 alleles and neurological adverse reactions to thalidomide. Further studies in larger numbers of patients may be needed to determine the roles of polymorphic CYP alleles in treatment outcome. VTE with the subsequent risk of pulmonary embolism (PE) is a major concern in the treatment of MM patients with thalidomide. Deep venous thrombosis (DVT) and/or PE occurs in only about 1–3% of patients receiving single-agent thalidomide for myeloma. The risk of thalidomide-induced thrombosis is highest in newly diagnosed patients when the drug is given in combination with dexamethasone, doxorubicin, or other chemotherapeutic drugs. The risk is elevated in the elderly and in patients with an underlying inherited or acquired thrombotic predisposition. The use of routine prophylactic warfarin or low molecular weight heparin or aspirin for all patients receiving thalidomide in combination with dexamethasone is currently under consideration. The susceptibility to the development of VTE in response to thalidomide therapy is likely to be influenced by both genetic and environmental factors. SNPs associated with thalidomide-related VTE were enriched in genes and pathways important in drug transport/metabolism, DNA repair, and cytokine balance.

Other Factors

Effects of liver or kidney dysfunction: The absorption and elimination of thalidomide are not significantly different in patients with hepatic dysfunction. Thalidomide is mainly hydrolyzed and passively excreted, and its pharmacokinetics are not expected to be altered in patients with impaired liver or kidney function. The inter- and intra-patient variability in liver or kidney dysfunction was low. There was no correlation between thalidomide clearance and renal function. Although clearance during dialysis is doubled, thalidomide dose need not be changed for patients with decreased kidney function. There is also no need for a supplementary dose due to hemodialysis. The serum concentration of thalidomide in MM patients with renal insufficiency was investigated in Japanese patients. The serum concentration of thalidomide 12 and 16 h after administration in patients with MM on hemodialysis (HD) taking 100–200 mg/day were similar with or without HD. The thalidomide concentration was not significantly increased by renal insufficiency. In this study, there is no correlation between the concentration of thalidomide and its clinical effect. In Japanese patients, the thalidomide dosage need not be modified for renal insufficiency or HD.

Effects of food on the pharmacokinetics of thalidomide: Although food often delays and/or decreases drug absorption, the absorption of a few drugs is increased by food. The effects of food on the oral pharmacokinetics of thalidomide and the relative bioavailability of two oral thalidomide formulations were determined by Teo et al. Five male and eight female healthy volunteers received a single oral dose of 200 mg thalidomide in capsule form under fasting and non-fasting conditions. A high-fat breakfast delayed the onset of absorption of thalidomide by 0.5–1.5 h.

Gender difference on the pharmacokinetics of thalidomide: Although there were no statistically significant differences in any of the pharmacokinetic parameters of thalidomide pharmacokinetics between men and women, tendencies toward gender differences in the pharmacokinetics of thalidomide were observed for some of the parameters. For example, women tended to have slightly larger Cmax and AUC values than men. These differences can be explained by the greater body weight of the male subjects. The mean half-life and mean residence time were also slightly larger for females than for males.

Differences in pharmacokinetic parameters among formulations: Terminal half-life showed two- to three-fold differences among tested formulations and is clear evidence for absorption rate limitations. Fujita et al. compared the dissolution profile and plasma thalidomide concentrations of Japanese and British capsules and Mexican tablets. The dissolution rate of the Japanese capsule was the fastest, followed by the British and Mexican formulations. The pharmacokinetic profiles of the Japanese and British capsules were similar, while the 100 mg Japanese thalidomide capsule showed a 1.6-fold higher maximum plasma concentration than the 200 mg Mexican thalidomide tablet, greatly shortened Tmax, and an apparent half life that was only one-third that of the Mexican tablet. Thus, pharmacokinetic changes may occur in plasma thalidomide concentration when switching between different formulations.

Conclusions

In conclusion, this review provided insights into the contribution of gene variations, drug-drug interactions, liver and kidney dysfunction, and thalidomide formulations to pharmacokinetic changes of thalidomide (Table 2). CYP2C19 poor metabolizer (PM) patients

Table 2. Effects of various factors on thalidomide pharmacokinetics in humans

<table>
<thead>
<tr>
<th>Factors</th>
<th>Thalidomide pharmacokinetics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>No significant difference</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>(slightly higher Cmax and AUC in females)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>No significant difference</td>
<td>29</td>
</tr>
<tr>
<td>Renal function</td>
<td>No significant difference</td>
<td>48–50</td>
</tr>
<tr>
<td>Hepatic function</td>
<td>No significant difference</td>
<td>47</td>
</tr>
<tr>
<td>Food</td>
<td>High-fat meal delayed the absorption of thalidomide</td>
<td>24</td>
</tr>
<tr>
<td>Transporter polymorphisms</td>
<td>Untransported (in vivo data)</td>
<td></td>
</tr>
<tr>
<td>CYP1A2 polymorphisms</td>
<td>No significant difference</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>(larger AUC in CYP1A2 genotype)</td>
<td></td>
</tr>
<tr>
<td>Formulations</td>
<td>Pharmacokinetic changes occur when switching to other formulations</td>
<td>51, 52</td>
</tr>
</tbody>
</table>
Tend to have high serum thalidomide concentrations and high risks of adverse drug effects, such as constipation, somnolence, and peripheral neuropathy. Further studies to clarify the mechanisms underlying the pharmacokinetic changes of thalidomide are required. Several next-generation antiepileptic drugs with improved tolerability profiles and reduced potential for drug interactions have been added to the therapeutic armamentarium. Thalidomide analogs termed immunomodulatory drugs (IMiDs, Fig. 2) have been developed that are more effective and have less toxicity than thalidomide. Thalidomide and its co-stimulatory IMiD analogs are currently being assessed in patients with advanced myeloma and some solid tumors, with promising effects.\(^9\) However, despite the promising effects of thalidomide on a broad range of serious diseases, further careful studies on the pharmacological and pharmacodynamic properties of thalidomide are necessary.\(^{10}\) Although thalidomide and IMiDs show similar biological activities, IMiDs are more potent than thalidomide and achieve responses at lower doses. Lenalidomide, a thalidomide derivative, has also been shown to have a different toxicity profile.\(^{11}\) Pharmacokinetic and clinical interactions between lenalidomide and other drugs seemed to occur, as in vitro data indicated that lenalidomide is a P-gp substrate.\(^{12}\)

Overall, these advances in studies of thalidomide pharmacokinetics have expanded the opportunities for individualization of drug therapy with antiepileptic drugs, to enhance effectiveness and minimize the risk of ADRs.

**References**


Pharmacokinetic Changes in Thalidomide Therapy


