Polymorphisms of CYP2C19 gene are associated with the efficacy of thalidomide-based regimens in multiple myeloma

Yonghua Li, Jian Hou, Hua Jiang, Dongxing Wang, Weijun Fu, Zhengang Yuan, Yubao Chen, Lili Zhou

All authors from department of Hematology, Changzheng Hospital, the second hospital affiliated to the Second Military Medical University, 415 Fengyang Rd., Shanghai 200003, PR China.

Manuscript received January 28, 2007.
Manuscript accepted June 27, 2007.

Correspondence:
Jian Hou, Department of Hematology, Changzheng Hospital, The Second Military Medical University, 415 Fengyang Rd., Shanghai 200003, PR China.
E-mail: houjian167@sohu.com

ABSTRACT

In this study, CYP2C19 genotypes were tested by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method in 92 patients with multiple myeloma (MM). Sixty-two patients were treated with thalidomide plus dexamethasone (Thal+Dex) and 30 with thalidomide combined with chemotherapy (Thal+CC). The overall response rate of extensive metabolizers (EMs) was statistically higher than that of poor metabolizers (PMs) (62.6% vs. 33.3%, $p<0.05$). Similar results were also observed in the Thal+Dex cohort. For the first time, our primary data suggested that the polymorphisms of CYP2C19 gene are associated with the efficacy of thalidomide based regimens in MM.

Key words: thalidomide, multiple myeloma, CYP2C19 genotype, efficacy.

Haematologica 2007; 92:1246-1249. DOI: 10.3324/haematol.11319

Thalidomide represents the new class of active agents in the treatment of multiple myeloma (MM).[1] Although it was believed to have antiangiogenic and immunomodulatory activities, the precise mechanisms by which thalidomide mediated its antimyeloma activity were not well defined and individual response to treatment varied. Recently, some studies have suggested that thalidomide metabolism was required for its antiangiogenic activity and antimyeloma efficacy.[2,3] Furthermore, it had been determined that CYP2C19 was primarily responsible for the formation of 5-hydroxythalidomide (5-OH) and cis-5'-hydroxythalidomide (cis-5'-OH) in humans.[4]

Genetic polymorphism of CYP2C19 has been extensively studied since the first report on poor metabolizers (PMs) of mephenytoin,[5] and it has been reported that PMs represent 12-23% of most Asian populations.[6]

As subjects with variant allele of CYP2C19 gene have a lower or absent phenotype, we suggest that patients with polymorphisms that do not allow the metabolism of thalidomide receive little benefit from thalidomide based regimens.

Design and Methods

Patients and treatment design

This study investigated 92 patients with MM who were treated with thalidomide based regimens in our hospital from June 2000 to October 2006. There were 62 males and 30 females with a median age of 56 years (range 29-81). According to the Salmon and Durie staging system,[7] 1 patient had stage I, 6 patients had stage II, and 85 patients had stage III. Of all, 28 were newly diagnosed and 64 were refractory or relapsed with a median number of 4 chemotherapy cycles (range 1-21) before thalidomide was given. Thalidomide was initially administered orally at low dose of 100 mg per day with gradual escalation to 400 mg per day or as tolerated. The therapeutic regimens were divided into two arms: (i) thalidomide combined with dexamethasone 20mg/m^2/d d1-4, d9-12, d17-20 for the first month and then for 4 days monthly (Thal+Dex); (ii) thalidomide plus combined chemotherapy including standard *MP, M2* or VAD regimen (Thal+CC). All patients were informed of the potential benefit and toxicities associated with the treatment and gave their written consent.

Assessment of response

All patients were evaluated for response every month after treatment. Response criteria was based on those of the European Group for Blood and Marrow Transplantation (EBMT).[8] Complete remission (CR) was defined as a negative immunofixation test for myeloma protein in serum and urine, the absence of soft-tissue plasmacytomas, no increase in the size of lytic bone lesions, and less than 5% plasma cells in the marrow with confirmation of response by all of these criteria 6 weeks later. Very good partial remission (VGPR), partial remission (PR) and minimal response (MR) were defined as reduction of serum M-protein more than 75%, 50-74% and 25-49%, respectively. Progressive disease (PD) was defined as either a more than 25% increase of M-protein in serum or urine or an increase in the size and number of lytic bone lesions or plasmacytomas. No change (NC) was defined as not meeting the criteria of either response or progressive disease. Bone marrow aspirate and biopsy were required for patients who achieved CR or demonstrated PD. Response rates refer to those patients achieving at least a partial response.

Genotype test

We examined only two variant sequences: a transition (+681, G to A) at codon 71 in exon 5 which produces an aberrant splice site (CYP2C19m^1) and a transition (+636, G to A) in exon 4 that results in a premature stop codon (CYP2C19m^2) because these 2 mutations account for more than 99% defective alleles in oriental populations.[9] The genomic DNA was extracted from peripheral blood mononuclear cells (PBMC) using the DNAzol reagent (Invitrogen Inc. Carlsbad, CA, USA). All samples were stored at -20° until use. CYP2C19 genotype was observed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods as previously described.[9] The normal CYP2C19 allele was referred to CYP2C19wt.

Statistical analysis

Comparisons of response rate to thalidomide based treatment between extensive metabolizers (EMs) and PMs were performed by χ^2 test. Statistical analysis was carried out using the SPSS13.0 software packages version. The minimal level of significance was $p<0.05$.

Results and Discussion

Genotype

The results of genotype tests in 92 patients revealed CYP2C19wt/wt in 39 patients (42.4%), CYP2C19wt/m^1 in 27 patients (29.3%), CYP2C19m^1/m^1 in 16 patients (17.4%), CYP2C19m^1/m^2 in 2 patients (2.2%), and CYP2C19wt/m^2 in 8 patients (8.7%). None of these patients had CYP2C19m^2/m^2. There were 18 PMs (CYP2C19m^1/m^1 and CYP2C19m^1/m^2) identified in our study, giving an incidence rate of 19.5%. This was similar to that in healthy Chinese population.[6] The remaining 74 patients were EMs including 39 homozygotes (CYP2C19wt/wt) and 35 heterozygotes (CYP2C19wt/m^1 and CYP2C19wt/m^2). Table 1 summarizes the baseline characteristics of PMs and EMs. The average dose of thalidomide PMs and EMs received was 248 and 256 mg/d, respectively ($p=0.783$, t test).

Table 1. Baseline patient characteristics by CYP2C19 genotype.

Characteristics	PMs		EMs	
	N.	%	N.	%
Sex, $p=0.295$				
Male	14	77.8	48	64.9
Female	4	22.2	26	35.1
Age, $p=0.157$				
≤ 65 years	15	83.3	49	66.2
> 65 years	3	16.7	25	33.8
Stage, $p=0.398$				
I	0	0	2	2.7
II	2	11.1	3	4.1
III	16	89.9	69	93.2
Isotype, $p=0.877$				
IgG	7	38.9	25	33.8
IgA	3	16.7	20	27.0
IgD	3	16.7	8	10.8
Light chains	4	22.2	16	21.6
Nonsecretory	1	5.6	5	6.8
Serum creatinine level, $p=0.759$				
<176.8 μmol/L	14	77.8	55	74.3
≥176.8 μmol/L	4	22.2	19	25.7
Prior therapy, $p=0.785$				
Newly diagnosed	5	27.8	23	31.1
Relapsed/refractory	13	72.2	51	68.9

EMs: extensive metabolizers; PMs: poor metabolizers.

Clinical response

According to EBMT criteria, CR was achieved in 7 patients (7.6%), VGPR in 12 patients (13%), and PR in 33 patients (35.9%), giving an overall response rate of 56.5% (52 out of 92 patients). Fourteen patients had MR, 14 patients NC, and 12 patients PD. EMs of CYP2C19 were mainly distributed in VGPR and PR, whereas PMs mainly in PR, NC, and PD. (Figure 1).

Association of CYP2C19 genotype with response to thalidomide based regimens

The overall response rate of EMs was statistically higher than that of PMs treated with thalidomide based regimens (62.6% vs. 33.3%, $p<0.05$). In the Thal+Dex cohort, the response rate of EMs was also statistically higher than that of PMs (60.8% vs. 27.3%, $p<0.05$). Similar results were observed in the Thal+CC group although there was no statistical difference (65.2% vs. 42.7%, $p>0.05$) (Table 2).

Table 2. Metabolism activity of CYP2C19 and response rate in 92 patients with multiple myeloma (MM) treated with thalidomide based regimens.

CYP2C19 activity	*Response* *nonresponders (n)*	*responders(n)*	*Response rate (%)*	*p value*
Thal+Dex				
EMs	20	31	60.8	
PMs	8	3	27.3	0.043
Thal+CC				
EMs	8	15	65.2	
PMs	4	3	42.7	0.29
Overall				
EMs	28	46	62.6	
PMs	12	6	33.3	0.027

EMs: extensive metabolizers; PMs: poor metabolizers; Thal+Dex: thalidomide plus dexamethasone; Thal+CC: thalidomide plus combined chemotherapy.

Many studies have shown that thalidomide requires microsomal CYP450 mediated biotransformation to exert its pharmacologic activity such as toxicity to lymphocytes, inhibition of cellular adhesion, alternation of cell morphology, and differentiation and antiangiogenesis.[2,10-12] Although the main transformation of thalidomide is spontaneous non-enzymatic hydrolysis, these breakdown products are not responsible for its activity.[13] Yaccoby *et al.* demonstrated that the presence of human liver tissue markedly increases thalidomide's antimyeloma efficacy using the myelomatous severe combined immunodeficiency-human (SCID-hu) model.[3] Others have reported that the CYP2C subfamily, especially CYP2C19 in humans, was involved in the metabolism of thalidomide at least in part, for the formation of 5-OH and cis-5'-OH.[4] We, therefore, investigated the association of CYP2C19 genotype with the antimyeloma effect of thalidomide. Our results show that the PMs had a lower response rate than EMs treated with thalidomide based regimens, although no statistical difference was observed in the Thal+CC cohort, possibly due to the small number of patients (n=30). The variation of CYP2C19 activity caused by its genetic polymorphism can alter the drug metabolism of many therapeutic agents such as mephenytoin, diazepam, imipramine, omeprazole, proguanil and certain barbiturates and may change their pharmacological activities.[6] It has been reported that, in pulse cyclophosphamide treatment of proliferative lupus nephritis, PMs had a higher probability of a poor renal response because of a reduction in the metabolic activation of cyclophosphamide.[14] As for thalidomide, these would be a very low concentration or absence of the active metabolites in patients with the PM genotype and they may receive little therapeutic benefit from thalidomide treatment. However, *in vitro* experiments demonstrated that even in the absence of metabolism, thalidomide also inhibited angiogenesis and affects cytokine expression in various cell types,[15-17] although high concentrations are required. It was, therefore, suggested that some metabolite catalyzed by CYP2C19 may have a greater antimyeloma activity than thalidomide itself or could enhance other intermediate

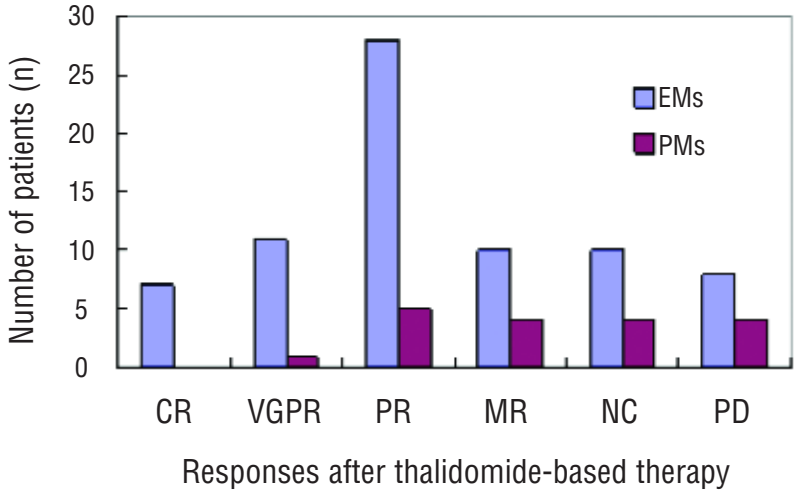

Figure 1. Distribution of patient response with different genotypes of CYP2C19 treated with thalidomide based therapy according to the European Group for Blood and Marrow Transplantation (EBMT) criteria. EMs of CYP2C19 were mainly distributed in VGPR and PR, while PMs mainly in PR, NC, and PD. EMs: extensive metabolizers; PMs: poor metabolizers; CR: complete response; VGPR: very good partial response; PR: partial response; MR: minimal response; NC: no change; PD: progressive disease.

products to exert their pharmacologic effect.

In addition, we had reported that advanced age had an adverse impact on response to thalidomide in MM.[18] However, there was no difference in distribution of age between EMs and PMs (*data not shown*). It was also demonstrated that the activity of CYP2C19 can decrease in some conditions such as advanced cancer which results in discordance between genotype and phenotype of this enzyme.[19] Furthermore, the activity of CYP2C19 can also to some extent be induced or inhibited by its substrate. It is still not known whether these factors have an effect on the antimyeloma efficacy of thalidomide in MM. These aspects were not investigated in this study because not all the necessary information was available for all patients. An appropriately designed prospective study will be required to further our understanding.

Author's contributions

LY and HJ designed the study. LY was responsible for the data management. LY and HJ contributed to the analysis and interpretation of the results and writing the manuscript draft. ZL and LY were responsible for the statistical analyses. JH, WD, FW, YZ and CY recruited patients in the data base. The manuscript was approved by all authors.

Conflict of Interest

The authors reported no potential conflicts of interest.

References

1. Singhal S, Mehta J, Desikan R, Ayers D, Roberson P, Eddlemon P, et al. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med 1999;341:1565-71.
2. Bauer KS, Dixon SC, Figg WD. Inhibition of angiogenesis by thalidomide requires metabolic activation, which is species-dependent. Biochem Pharmacol 1998;55:1827-34.
3. Yaccoby S, Johnson CL, Mahaffey SC, Wezeman MJ, Barlogie B, Epstein J. Antimyeloma efficacy of thalidomide in the SCID-hu model. Blood 2002;100:4162-8.
4. Ando Y, Fuse E, Figg WD. Thalidomide metabolism by the CYP2C subfamily. Clin Cancer Res 2002; 8: 1964-73.
5. Goldstein JA, de Morais SM. Biochemistry and molecular biology of the human CYP2C subfamily. Pharmacogenetics 1994;4:285-99.
6. Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol 2001;52:349-55.
7. Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer 1975;36:842-54.
8. Blade J, Samson D, Reece D, Apperley J, Bjorkstrand B, Gahrton G, et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. Br J Haematol 1998;102:1115-23.
9. Goldstein JA, Blaisdell J. Genetic tests which identify the principal defects in CYP2C19 responsible for the polymorphism in mephenytoin metabolism. Methods Enzymol 1996;272: 210-8.
10. Braun AG, Harding FA, Weinreb SL. Teratogen metabolism: thalidomide activation is mediated by cytochrome P-450. Toxicol Appl Pharmacol 1986;82:175-9.
11. Hatfill SJ, Fester ED, de Beer DP, Bohm L. Induction of morphological differentiation in the human leukemic cell line K562 by exposure to thalidomide metabolites. Leuk Res 1991;15:129-36.
12. Nicolai S, Sies H, Stahl W. Stimulation of gap junctional intercellular communication by thalidomide and thalidomide analogs in human skin fibroblasts. Biochem Pharmacol 1997;53:1553-7.
13. Schumacher H, Smith RL, Williams RT. The metabolism of thalidomide: the spontaneous hydrolysis of thalidomide in solution. Br J Pharmacol Chemother 1965;25:324-37.
14. Takada K, Arefayene M, Desta Z, Yarboro CH, Boumpas DT, Balow JE, et al. Cytochrome P450 pharmacogenetics as a predictor of toxicity and clinical response to pulse cyclophosphamide in lupus nephritis. Arthritis Rheum 2004;50:2202-10.
15. Hideshima T, Chauhan D, Shima Y, Raje N, Davies FE, Tai YT, et al. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood 2000;96:2943-50.
16. Vacca A, Scavelli C, Montefusco V, Di Pietro G, Neri A, Mattioli M, et al. Thalidomide downregulates angiogenic genes in bone marrow endothelial cells of patients with active multiple myeloma. J Clin Oncol 2005;23:5334-46.
17. Rowland TL, McHugh SM, Deighton J, Dearman RJ, Ewan PW, Kimber I. Differential regulation by thalidomide and dexamethasone of cytokine expression in human peripheral blood mononuclear cells. Immunopharmacology 1998;40:11-20.
18. Li Y, Hou J, Wang D, Fu W, Yuan Z, Chen Y, et al. Prognostic factors for the efficacy of thalidomide in the treatment of multiple myeloma: A clinical study of 110 patients in China. Leuk Lymphoma 2006;47: 2593-600.
19. Williams ML, Bhargava P, Cherrouk I, Marshall JL, Flockhart DA, Wainer IW. A discordance of the cytochrome P450 2C19 genotype and phenotype in patients with advanced cancer. Br J Clin Pharmacol 2000;49:485-8.