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SHORT COMMUNICATION

Lenalidomide: in vitro evaluation of the metabolism and assessment of cytochrome P450 inhibition and induction

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Abstract
Purpose To assess the potential for drug–drug interactions between lenalidomide and substrates and inhibitors of cytochrome P450 (CYP) isozymes.
Methods In vitro metabolism of lenalidomide by human liver microsomes, recombinant human CYPs and human hepatocytes was evaluated. The inhibitory and inductive effects of lenalidomide on the CYP activities were evaluated in human liver microsomes and cultured human hepatocytes, respectively.
Results In vitro incubation of lenalidomide with human liver microsomes, recombinant-CYP isozymes, and human hepatocytes did not result in Phase I or Phase II metabolism, confirming the low propensity of lenalidomide for metabolism in vivo in humans. In vitro, lenalidomide did not inhibit CYP isozymes in human liver microsomes and did not induce CYP activities in cultured human hepatocytes.
Conclusions Lenalidomide is not a substrate, inhibitor, or inducer of CYP group of enzymes; clinically relevant pharmacokinetic drug–drug interactions are unlikely to occur between lenalidomide and co-administered CYP substrates or inhibitors.

Keywords Lenalidomide · Cytochrome P450 · CYP inhibition · CYP induction · Drug–drug interactions

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Introduction

Lenalidomide (CC-5013) is a thalidomide analog possessing immunomodulatory, antiproliferative, and antiangiogenic properties [1, 2]. Lenalidomide is used for the treatment of myelodysplastic syndromes with certain cytogenetic abnormalities (5q-syndrome) [3, 4] and multiple myeloma [5–7]. Currently, clinical trials are ongoing to evaluate the efficacy and safety of lenalidomide in patients with various B cell malignancies and solid tumors [8–10].

Cytochrome P450 is a superfamily of mixed function oxidases that are responsible for the metabolism of many drugs including oral contraceptives, dexamethasone, and many anticancer agents such as taxanes. Some of these enzymes are polymorphically expressed (e.g., CYP2D6, CYP2C19) that result in wide variability of pharmacokinetics of their substrate drugs. Cytochrome P450 isozymes are subject to inhibition via competitive or other mechanisms by drugs such as ketoconazole, ritonavir and clarithromycin, leading to clinically relevant increases in the exposure of the affected drug. Further, some of the CYP isoforms are also subject to induction by xenobiotics via activation of nuclear hormone receptors, with a consequent result of decreased exposure of the affected compound leading to therapeutic failure (e.g., rifampicin and oral contraceptive steroids) or toxicological implications due to higher levels of an undesired metabolite [11, 12].

For a new molecular entity, it is important to assess the role of CYP enzymes in its overall clearance, and the likely inhibitory or inductive effects of the new molecular entity on the metabolic reactions catalyzed by CYP isozymes. Anticancer therapeutic regimens often involve polypharmacy comprising of multiple therapeutic agents and/or palliative therapies. It is likely that lenalidomide will be administered with other therapeutic agents in a clinical

setting. For example, for the treatment of multiple myeloma, lenalidomide is administered in combination with dexamethasone, a substrate and a weak inducer of CYP3A4/5 isoforms [13, 14]. This study was conducted to evaluate the potential for pharmacokinetic drug–drug interactions with lenalidomide as a substrate, inhibitor or inducer of CYP enzymes.

Materials and methods

Radiolabeled lenalidomide was synthesized by Amersham Pharmacia Biotech, Amersham, Buckinghamshire, UK (2.07 GBq/mmol; 56 mCi/mmol). Non-radiolabeled lenalidomide was synthesized by Evotec Inc., Abingdon, UK, for Celgene Corporation.

Metabolism by human liver microsomes and recombinant human CYPs

A mixed pool of human liver microsomes (46 donors) was purchased from Totam Biologicals, Peterborough, UK. Recombinant human CYP isoforms CYP1A2, CYP2A6, CYP2C8, CYP2C9 (Arg_{144}), CYP2C19, CYP2D6 (Val_{374}), CYP2E1, and CYP3A4 (Supersomes®, microsomes from insect cells infected with baculovirus transfected with specific CYP isoforms, including wildtype) were purchased from BD Gentest Corporation, Woburn, MA. Incubations comprised of Tris buffer (50 mM, pH 7.4), ^{14}C-lenalidomide (10 μM final concentration), and human microsomal protein (1 mg/mL) or recombinant human CYP isozyme (100 pmol enzyme/mL). The incubation mixture was pre-incubated at 37°C for 5 min prior to the initiation of metabolic reaction with 2 mM β-Nicotinamide adenine dinucleotide hydrogen phosphate (β-NADPH), in duplicate, alongside a negative control incubated without NADPH. The metabolic reaction was terminated after 60 min, with the addition of an equal volume of acetonitrile. Following centrifugation (13,000 rpm; 5 min) to precipitate protein, supernatants were analyzed by reversed-phase HPLC with an online flow scintillation analyzer. A parallel set of incubations were conducted with positive control substrates to confirm the metabolic capacity of the source enzymes.

Metabolism by human hepatocytes

Fresh human hepatocytes, obtained from the UK Human Tissue Bank (UKHTB), Leicester, UK, were isolated from tissue samples removed from a living donor undergoing liver resection. ^{14}C-Lenalidomide (1, 5, or 25 μM) was incubated with hepatocytes (2×10^6 cells/mL) in incubation medium at 37°C in duplicate. After incubating for 0, 1, 2, 4, or 6 h, the metabolic reactions were terminated by the addition of methanol and vortexing. Control incubations were performed in the absence of hepatocytes. The supernatants (obtained after centrifugation of the incubation mixtures at 13,000 rpm for 10 min) were analyzed by reversed-phase HPLC with an on-line flow scintillation analyzer. To assess the metabolic capacity of the human hepatocytes used in this assay, parallel incubations were conducted with ^{14}C-7-ethoxy-coumarin (50 μM, positive control).

Inhibitory effect on cytochromes P450

Pooled human liver microsomes (16 donors) were purchased from Totam Biologicals Ltd, Northampton, UK. ^{14}C-testosterone, ^{14}C-lauric acid, ^{14}C-*S*-mephenytoin, and ^{14}C-tolbutamide were obtained from Amersham Pharmacia Biotech (Aylesbury, Bucks, UK). All other reagents were obtained from Sigma Chemical Company Ltd (Poole, Dorset, UK), Aldrich Chemical Company Ltd (Gillingham, Dorset, UK), Ultrafine Chemicals (Manchester, UK), and Steraloids Ltd (New Barnet, Herts, UK). Except for quinidine, all other isoform-specific inhibitors of CYP isoforms, furafylline (CYP1A2), sulfaphenazole (CYP2C9), tranylcypromine (CYP2C19), disulfiram (CYP2E1), and miconazole (CYP3A4) were dissolved in acetonitrile at a level 100 times the final concentration required in the incubation mixture; quinidine (CYP2D6) was dissolved in Tris buffer (50 mM, pH 7.4) at a concentration ten times that required in the incubation mixture. Incubation conditions for evaluating CYP inhibition are summarized in the Table 1.

Inductive effect on cytochromes P450

Acetaminophen, bupropion HCl, diclofenac, 4′-hydroxydiclofenac, 6β-hydroxytestosterone, β-NADP, omeprazole, phenobarbital, phenacetin, rifampin, and trypan blue were purchased from Sigma Aldrich Chemical Co. (St Louis, MO, USA); hydroxybupropion was purchased from BD Gentest (San Jose, CA, USA), *S*-mephenytoin was purchased from Toronto Research Chemicals (North York, ON, Canada), and (±)-4′-hydroxy-mephenytoin was purchased from Ultrafine Chemicals (a division of Sigma–Aldrich Chemical Co., St Louis, MO, USA). Hepatocytes were isolated and cultured according to previously described methods [15–17]. The viability of hepatocytes was assessed by the trypan blue exclusion method. Cultures were allowed to adapt to the culture environment for 3 days with daily media replacement. Hepatocyte cultures were treated twice daily for three consecutive days, with medium containing 0.1% dimethyl sulfoxide (DMSO) (vehicle, negative control), one of three concentrations of lenalidomide (1, 3, or 10 μM), or one of three known human CYP enzyme inducers (100 μM omeprazole, 750 μM phenobarbital, 10 μM rifampin). At the end of the treatment period,

Table 1 Inhibitory effect of lenalidomide on isoform specific cytochrome P450 activities in human liver microsomes

CYP isoform-specific probe activity and probe concentration	Incubation time (min)	Analytical method	Lenalidomide or positive control inhibitor conc. (μM)	Percent inhibition
CYP1A2: Ethoxyresorufin *O*-dethylation (1 μM)	4 (Continuous monitoring)	Fluorescence λ_{ex} = 530 nm λ_{em} = 585 nm	Lenalidomide (10)	NI[a]
			Lenalidomide (100)	NI[a]
			Furafylline (50)[b]	90.0
CYP2C9: Tolbutamide methyl hydroxylation (100 μM)	30	HPLC-FSA[c]	Lenalidomide (10)	9.6
			Lenalidomide (100)	12.6
			Sulfaphenazole (20)	86.0
CYP2C19: *S*-Mephenytoin 4′-hydroxylation (100 μM)	30	HPLC-FSA[c]	Lenalidomide (10)	NI[a]
			Lenalidomide (100)	NI[a]
			Tranylcypromine (20)	50.6
CYP2E1: Lauric acid 11-hydroxylation (100 μM)	30	HPLC-FSA[c]	Lenalidomide (10)	23.2
			Lenalidomide (100)	20.1
			Disulfiram (200)	94.5
CYP2D6: Bufarolol 1-hydroxylation (10 μM)	30	HPLC-fluorescence detection	Lenalidomide (0.1)	22.5
			Lenalidomide (0.3)	23.5
			Lenalidomide (1)	20.7
			Lenalidomide (3)	30.4
			Lenalidomide (10)	29.7
			Lenalidomide (30)	38.2
			Lenalidomide (100)	22.1
			Quinidine (0.5)	57.4
CYP3A4/5: Testosterone 6β-hydroxylation (100 μM)	10	HPLC-FSA[c]	Lenalidomide (0.1)	NI[a]
			Lenalidomide (0.3)	NI[a]
			Lenalidomide (1)	14.2
			Lenalidomide (3)	8.9
			Lenalidomide (10)	4.0
			Lenalidomide (30)	0.8
			Lenalidomide (100)	3.5
			Miconazole (2)	74.0

[a] NI no inhibition

[b] Pre-incubated in the presence of NADPH prior to the addition of probe substrate

[c] HPLC-FSA: high-performance liquid chromatography with flow scintillation analysis

microsomal samples were prepared, and stored at −80°C until analysis. Microsomal incubations were carried out at 37°C with the following isoform-specific probe activities at the concentrations indicated: phenacetin *O*-deethylation for CYP1A2 (80 μM phenacetin, 30-min incubation), bupropion hydroxylation for CYP2B6 (500 μM bupropion, 30-min incubation), diclofenac 4′-hydroxylation for CYP2C9 (100 μM diclofenac, 10-min incubation), *S*-mephenytoin 4′-hydroxylation for CYP2C19 (400 μM *S*-mephenytoin, 30-min incubation), and testosterone 6β-hydroxylation for CYP3A4/5 (250 μM testosterone, 10-min incubation) were used as isoform-specific catalytic activities. All incubations were conducted in duplicate wells at 37 ± 1°C in incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), EDTA (1 mM), NADP (1 mM), glucose-6-phosphate (5 mM), and glucose-6-phosphate dehydrogenase (1 Unit/mL). Reactions were initiated by addition of the NADPH-generating system, and were terminated after the appropriate incubation time by the addition of acetonitrile. Precipitated protein was removed by centrifugation (920×*g* for 10 min at 10°C), and supernatant fractions were analyzed by HPLC/MS/MS for the respective metabolites.

Results

Metabolism by human liver microsomes

When ^{14}C-lenalidomide was incubated with human liver microsomes or recombinant human CYP isozymes, there was no metabolism observed when compared to the corresponding negative control incubations conducted without enzyme source.

Metabolism by human hepatocytes

As anticipated, positive control substrate, ^{14}C-7-ethoxycoumarin was metabolized to 7-hydroxy-courmarin and subsequently glucuronidated and sulfated, confirming the metabolic capacity of the hepatocytes used in this assay. No metabolism of ^{14}C-lenalidomide was observed in human

hepatocytes after 6 h incubation compared to media controls. In negative control incubations as well as in incubations with hepatocytes, products were observed that resulted from non-enzymatic hydrolysis of the glutarimide ring of ^{14}C-lenalidomide.

Inhibitory effects on cytochromes P450

The effect of lenalidomide on isoforms-specific CYP activities in human liver microsomes is summarized in Table 1. As expected, all positive control inhibitors produced inhibitory effects on the respective catalytic activities. No significant inhibition of CYP1A2-dependent ethoxyresorufin *O*-dealkylase (<6%), CYP2C9-dependent tolbutamide methyl-hydroxylase activity (<13%), CYP2C19-dependent *S*-mephenytoin 4′-hydroxylase activity, or CYP2E1-dependent lauric acid 11-hydroxylase activity (<24%) was observed for either 10 or 100 μM lenalidomide. There was no marked concentration-dependency of CYP2D6 inhibition in the concentration range of 0.1–100 μM lenalidomide. A similar lack of inhibition by lenalidomide of CYP3A4/5 catalytic activity was also observed.

Inductive effects on cytochromes P450

At the time of isolation, the viability of each hepatocyte preparation was between 76.9 and 82.3%. During the adaptation followed by 72 h incubation period, human cultured hepatocytes were cuboidal and contained intact cell membranes and granular cytoplasm with one or two centrally located nuclei. Enzymatic activities (pmol/mg protein/min) and fold-over DMSO control data are presented in Table 2. As anticipated, the positive control inducers produced marked elevations of the respective isoforms-specific catalytic activities. Treatment of cultured human hepatocytes with up to 10 μM lenalidomide, twice daily for three consecutive days, did not result in increased catalytic activities of any of the CYP enzymes examined, namely CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4/5.

Discussion

Results of this study demonstrate that in vitro lenalidomide is not a substrate for CYP isozymes. There is no evidence to suggest that lenalidomide is subject to direct conjugative metabolism either. Lenalidomide undergoes hydrolysis in buffer and human plasma at neutral pH at a moderate rate, with an in vitro half life of approximately 24 and 8 h, respectively, due to hydrolytic cleavage of glutarimide amide bonds (unpublished data). In humans, excretion of the unchanged parent in urine is the predominant clearance mechanism of lenalidomide, accounting for approximately

Table 2 Effect of lenalidomide on isoform specific cytochrome P450 enzymatic activities in cultured human hepatocytes

		Enzymatic activity (pmol/mg protein/min) (fold-over DMSO control) (mean ± SD; $n = 3$)				
Treatment	Conc.	Phenacetin *O*-dealkylation (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Diclofenac 4′-hydroxylation (CYP2C9)	*S*-Mephenytoin 4′-hydroxylation (CYP2C19)	Testosterone 6*β*-hydroxylation (CYP3A4/5)
DMSO	0.1% (v/v)	49.2 ± 6.9	52.2 ± 11.9	1,130 ± 350	21.7 ± 13.9	5,540 ± 250
Lenalidomide	1 μM	52.4 ± 8.6 (1.07 ± 0.11)	57.6 ± 5.2 (1.13 ± 0.20)	1,200 ± 310 (1.08 ± 0.06)	24.0 ± 18.3 (1.06 ± 0.19)	6,410 ± 150 (1.16 ± 0.06)
Lenalidomide	3 μM	45.6 ± 10.2 (0.92 ± 0.08)	46.0 ± 15.2 (0.88 ± 0.19)	1,080 ± 390 (0.95 ± 0.11)	23.3 ± 16.2 (1.02 ± 0.13)	5,400 ± 430 (0.98 ± 0.10)
Lenalidomide	10 μM	50.4 ± 9.8 (1.02 ± 0.08)	50.3 ± 8.1 (0.99 ± 0.14)	1,130 ± 320 (1.00 ± 0.05)	25.0 ± 17.3 (1.10 ± 0.11)	5,810 ± 430 (1.05 ± 0.12)
Omeprazole	100 μM	1,890 ± 1,260 (37.1 ± 20.1)	553 ± 324 (10.0 ± 4.9)	1,490 ± 270 (1.37 ± 0.22)	11.8 ± 5.0 (0.67 ± 0.30)	4,920 ± 1,030 (0.89 ± 0.22)
Phenobarbital	750 μM	104 ± 23 (2.11 ± 0.20)	892 ± 739 (15.6 ± 10.6)	1,680 ± 680 (1.47 ± 0.18)	46.1 ± 32.4 (2.92 ± 2.00)	10,400 ± 3,800 (1.87 ± 0.620
Rifampin	10 μM	108 ± 27 (2.26 ± 0.86)	488 ± 228 (8.99 ± 2.41)	2,350 ± 520 (2.14 ± 0.30)	181 ± 175 (8.88 ± 6.02)	15,400 ± 4,700 (2.79 ± 0.89)

84% of the dose [18]. These data suggests that metabolism plays a minor role in the overall disposition of lenalidomide. Overall, in vivo data confirm the in vitro findings of this study, which is lack of Phase I and Phase II metabolism of lenalidomide to a considerable extent. Since lenalidomide is not subject to oxidative metabolism by CYPs, coadministration of potent CYP inhibitors such as ketoconazole, fluconazole, fluoxamine, and quinidine are not likely to result in clinically relevant changes in lenalidomide exposure.

The results of this study also demonstrate that lenalidomide does not inhibit or induce CYP enzymes in vitro. The peak plasma concentrations of lenalidomide at therapeutic doses are ≤ 2 μM [18], which are considerably lower than the highest concentrations evaluated in this in vitro study. Hence, lenalidomide is not anticipated to precipitate clinically relevant pharmacokinetic drug–drug interactions when coadministered with CYP substrates such as oral contraceptive steroids and warfarin. In a clinical drug–drug interaction study, lenalidomide did not significantly affect warfarin pharmacokinetics (Lenalidomide Product Insert, Celgene Corporation), consistent with the lack of inhibition or induction of CYP2C9 activity in vitro by lenalidomide.

Conclusion

In summary, lenalidomide is not a substrate, inhibitor, or inducer of CYP group of enzymes. Hence, clinically relevant pharmacokinetic drug–drug interactions are not likely with lenalidomide either as an object when coadministered with CYP inhibitors or as a precipitant when coadministered with CYP substrates.

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