Thalidomide Metabolism and Metabolites

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By

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ABSTRACT

Thalidomide, renowned for causing birth defects in the late 1950s when used for the relief of morning sickness, has attracted new interest for the treatment of inflammatory conditions such as erythema nodosum leprosum and human malignancies such as multiple myeloma. Different species have different sensitivities to thalidomide that could be related to differences in its metabolism. In this study, methodologies using liquid chromatography-mass spectrometry were developed to identify thalidomide metabolites formed *in vivo* and *in vitro* in liver microsomes from mice, rabbits and humans, firstly to seek explanations for inter-species differences in sensitivity, and secondly to determine whether thalidomide or its metabolite(s) is the active agent.

Four hydrolysis products were detected in plasma and urine samples from multiple myeloma patients (MMPs) on thalidomide therapy, and mice and rabbits after oral administration of thalidomide. Six hydroxylated metabolites were detected in mice and rabbits, but not in plasma and urine from MMPs. *In vitro* studies confirmed that murine and rabbit liver microsomes catalysed the hydroxylation of thalidomide efficiently, but significant production of hydroxylation of thalidomide was not observed using human liver microsomes. The degree of hydroxylation both *in vivo* and *in vitro* was highest in mice and lowest in humans with rabbits in between. It is unlikely that hydroxylated metabolites are responsible for the effects of thalidomide in the treatment of multiple myeloma, since they were not present in quantifiable amounts in patients who were responding to the treatment. The three major hydrolysis products that were detected in patients were compared with thalidomide for their ability to inhibit tube formation in an *in vitro* angiogenesis assay, to inhibit TNF production induced with LPS in human peripheral blood leucocytes, and to modulate DMXAA-induced TNF production and antitumour activity in mice. One of the three, *N*-(*o*-carboxybenzoyl)glutamic acid imide (CG) was found to be as active as thalidomide in all the assays at concentrations (1-2 μg/ml) that are achievable in MMPs. Since CG has been shown by other laboratories to be non-teratogenic, the studies in this

thesis indicate that CG would be a more favourable, non-teratogenic approach to cancer therapy compared with thalidomide.

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5-OH Th	5-hydroxythalidomide
5'-OH CG	5'-hydroxy-*N*-(*o*-carboxybenzoyl)glutamic acid imide
5'-OH Th	5'-hydroxythalidomide
α-MEM	α-minimal essential medium
μg	microgram
μl	microlitre
μM	micromolar
ACN	acetonitrile
amu	atomic molecular unit
APCI	atmospheric pressure chemical ionisation
AU	arbitrary unit(s)
AUC	area under the concentration-time curve
bFGF	basic fibroblast growth factor
BMSC	bone marrow stromal cell
C_{max}	maximal drug concentration following administration
CG	*N*-(*o*-carboxybenzoyl)glutamic acid imide
CL_{int}	intrinsic clearance
Cox	cyclooxygenase
Cl/F	apparent clearance rate
CV	coefficient of variation
CYP	cytochrome P450 enzymes
DMSO	dimethylsulphoxide
DMXAA	5,6-dimethylxanthenone-4-acetic acid
ELISA	enzyme linked immunosorbent assay
FBS	fetal bovine serum
g	gram
g	gravity
h	hour

HCl	hydrochloric acid
HIV	human immunodeficiency virus
HPBL	human peripheral blood leucocytes
HPCD	2-hydroxypropyl-β-cyclodextrin
HPLC	high performance liquid chromatography
HUVEC	human umbilical vein endothelial cell
ICAM	intercellular cell adhesion molecule
IFN	interferon
IGF	insulin-like growth factor
IL	interleukin
i.p.	intraperitoneal
i.v.	intravenous
KCl	potassium chloride
K_M	Michaelis-Menten constant
kg	kilogram
LC-MS	liquid chromatography-mass spectrometry
LPS	lipopolysaccharide
mAU	milli arbitrary unit(s)
mg	milligram
min	minute
ml	milliliter
mM	millimolar
MMP	multiple myeloma patient
MRI	magnetic resonance imaging
MS	mass spectrometry
MSD	mass spectral detection
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NADPH	β-nicotinamide adenine dinucleotide phosphate reduced form
NF-κB	nuclear factor-κB
NMR	nuclear magnetic resonance
PBS	phosphate buffered saline

PG	phthaloylglutamine
PiG	phthaloylisoglutamine
p.o.	oral
SEM	standard error of mean
SIM	single ion monitoring
$t_{1/2}$	drug half-life
T_{max}	time when C_{max} is achieved
TCA	trichloroacetic acid
Thal	thalidomide
TIC	total ion current
TNF	tumour necrosis factor-α
UDPG	uridine diphosphate glucuronide
UDPG-transferase	uridine diphosphate glucuronosyl transferase
UV	ultraviolet
V	velocity of the reaction
V_{max}	maximum velocity of the reaction
V/F	volume of distribution
v/v	volume/volume
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor