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Thalidomide Metabolism and Metabolites

A thesis submitted to the University of Auckland
in fulfilment of the requirements for the degree of
Doctor of Philosophy

By

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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.
ACKNOWLEDGMENTS

Constructing a PhD thesis is like building a house. I was very lucky to have a group of great “architects”, my supervisors, to guide me and supply me with a “blue print” and “building materials”.

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years. We helped and cooperated with each other to carry out tough experiments, and produced a paper together, which has been submitted to *Clinical Cancer Research*.

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<table>
<thead>
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<tbody>
<tr>
<td>5-OH Th</td>
<td>5-hydroxythalidomide</td>
</tr>
<tr>
<td>5’-OH CG</td>
<td>5’-hydroxy-N-(o-carboxybenzoyl)glutamic acid imide</td>
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<tr>
<td>5’-OH Th</td>
<td>5’-hydroxythalidomide</td>
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<td>α-MEM</td>
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</tr>
<tr>
<td>ACN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>amu</td>
<td>atomic molecular unit</td>
</tr>
<tr>
<td>APCI</td>
<td>atmospheric pressure chemical ionisation</td>
</tr>
<tr>
<td>AU</td>
<td>arbitrary unit(s)</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the concentration-time curve</td>
</tr>
<tr>
<td>bFGF</td>
<td>basic fibroblast growth factor</td>
</tr>
<tr>
<td>BMSC</td>
<td>bone marrow stromal cell</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>maximal drug concentration following administration</td>
</tr>
<tr>
<td>CG</td>
<td>N-(o-carboxybenzoyl)glutamic acid imide</td>
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<tr>
<td>CL&lt;sub&gt;int&lt;/sub&gt;</td>
<td>intrinsic clearance</td>
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<td>cyclooxygenase</td>
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<td>enzyme linked immunosorbent assay</td>
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<td>g</td>
<td>gravity</td>
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<td>h</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
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</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HPBL</td>
<td>human peripheral blood leucocytes</td>
</tr>
<tr>
<td>HPCD</td>
<td>2-hydroxypropyl-β-cyclodextrin</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HUVEC</td>
<td>human umbilical vein endothelial cell</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular cell adhesion molecule</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>IGF</td>
<td>insulin-like growth factor</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>KCl</td>
<td>potassium chloride</td>
</tr>
<tr>
<td>$K_M$</td>
<td>Michaelis-Menten constant</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>mAU</td>
<td>milli arbitrary unit(s)</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>ml</td>
<td>milliliter</td>
</tr>
<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>MMP</td>
<td>multiple myeloma patient</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MSD</td>
<td>mass spectral detection</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide</td>
</tr>
<tr>
<td>NADPH</td>
<td>β-nicotinamide adenine dinucleotide phosphate reduced form</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor-κB</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>--------------</td>
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</tr>
<tr>
<td>PG</td>
<td>phthaloylglutamine</td>
</tr>
<tr>
<td>PiG</td>
<td>phthaloylisoglutamine</td>
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<tr>
<td>p.o.</td>
<td>oral</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of mean</td>
</tr>
<tr>
<td>SIM</td>
<td>single ion monitoring</td>
</tr>
<tr>
<td>t$_{1/2}$</td>
<td>drug half-life</td>
</tr>
<tr>
<td>T$_{max}$</td>
<td>time when C$_{max}$ is achieved</td>
</tr>
<tr>
<td>TCA</td>
<td>trichloroacetic acid</td>
</tr>
<tr>
<td>Thal</td>
<td>thalidomide</td>
</tr>
<tr>
<td>TIC</td>
<td>total ion current</td>
</tr>
<tr>
<td>TNF</td>
<td>tumour necrosis factor-$\alpha$</td>
</tr>
<tr>
<td>UDPG</td>
<td>uridine diphosphate glucuronide</td>
</tr>
<tr>
<td>UDPG-transferase</td>
<td>uridine diphosphate glucuronosyl transferase</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>V</td>
<td>velocity of the reaction</td>
</tr>
<tr>
<td>V$_{max}$</td>
<td>maximum velocity of the reaction</td>
</tr>
<tr>
<td>V/F</td>
<td>volume of distribution</td>
</tr>
<tr>
<td>v/v</td>
<td>volume/volume</td>
</tr>
<tr>
<td>VCAM</td>
<td>vascular cell adhesion molecule</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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