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Abstract

Opioid dependence is a significant public health problem. Whilst long-term opioid maintenance is the most cost-effective approach for treating opioid dependence, the safe and effective use of substitution opioids like methadone and buprenorphine is complicated by their narrow therapeutic indices and a considerable, as yet unexplained, interindividual variability in their dose-effect relationships. Since there is evidence that the P-glycoprotein efflux transporter may influence the plasma pharmacokinetics and CNS distribution of opioids, it was hypothesised that genetic variability in the \textit{ABCB1} gene (encoding P-glycoprotein) could play a major role in the interindividual variability in opioid maintenance treatment response. Therefore, the primary aim of this thesis was to investigate \textit{ABCB1} genetic variability as a determinant of opioid requirements during maintenance therapy, as well as treatment outcome. This thesis also set out to identify the relationship between \textit{ABCB1} genetic variability and the risk of illicit opioid use and dependence, as well as develop new methods for investigating the dynamic interactions between \textit{ABCB1} genetic variability, P-glycoprotein expression/function and opioid exposure.

For the first major study of this thesis, opioid-dependent methadone maintenance treatment (MMT, \(n = 78\)) and buprenorphine maintenance treatment (BMT, \(n = 30\)) subjects, as well as non-opioid-dependent healthy controls (\(n = 98\)), were retrospectively genotyped and haplotyped for 5 common single nucleotide polymorphisms (SNPs) of \textit{ABCB1} (A61G, G1199A, C1236T, G2677T and C3435T). Whilst no link was observed between \textit{ABCB1} genetic variability and the risk of opioid dependence, the wild-type AGCGC (61A-1199G-1236C-2677G-3435C) haplotype was associated with significantly higher maintenance opioid requirements among both MMT and BMT subjects. In addition, MMT subjects carrying one of the variant haplotypes, AGCTT, required significantly less methadone, presumably due to a decreased P-gp activity at the blood-brain-barrier. Interestingly, a second retrospective study of a specific cohort of 21 (very) high-dose (\(\geq 180\) mg/day) MMT subjects could not replicate
these findings, suggesting that dose range and/or clinic policy may be important factors influencing the clinical significance of ABCB1 genetic variability.

The third major study of this thesis incorporated the development and validation of new methods for quantifying ex vivo P-glycoprotein expression (mRNA and protein) and function in specific lymphocyte subsets (CD4+, CD56+ and CD8+) of healthy and opioid-dependent subjects, with the aim of determining the combined effects of ABCB1 genetic variability and opioid exposure on P-glycoprotein function. Applying these new methods in a pilot study of 6 MMT subjects, CD4+ lymphocyte ABCB1 mRNA and P-glycoprotein expression were found to be positively associated with methadone requirements, and were lowest in the only subject homozygous for the AGCTT haplotype (providing potential mechanistic support for the link between AGCTT haplotypes and low MMT dose requirements).

Therefore, this thesis provides the first evidence that ABCB1 haplotypes contribute to variability in substitution opioid requirements. However, ABCB1 genetic variability should not be considered alone, and a combined interpretation of multiple genetic and environmental factors will be required to provide a more complete picture of the factors governing the successful treatment of opioid dependence.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any University or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Daniel T Barratt

18 August 2010
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Publications in support of this thesis

Original research


Invited review

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-MAM</td>
<td>6-monoacetylmorphine</td>
</tr>
<tr>
<td>A&gt;B</td>
<td>Apical-to-basal permeability</td>
</tr>
<tr>
<td>A\textsubscript{260}</td>
<td>Absorbance at 260 nm</td>
</tr>
<tr>
<td>A\textsubscript{280}</td>
<td>Absorbance at 280 nm</td>
</tr>
<tr>
<td>AAG</td>
<td>$\alpha_1$-acid glycoprotein</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the concentration-time curve</td>
</tr>
<tr>
<td>B&gt;A</td>
<td>Basal-to-apical permeability</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-brain-barrier</td>
</tr>
<tr>
<td>BCA</td>
<td>Bicinchoninic acid</td>
</tr>
<tr>
<td>BMT</td>
<td>Buprenorphine maintenance treatment</td>
</tr>
<tr>
<td>bp</td>
<td>Base pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CL/F</td>
<td>Oral clearance</td>
</tr>
<tr>
<td>CL\textsubscript{R}</td>
<td>Renal clearance</td>
</tr>
<tr>
<td>C\textsubscript{max}</td>
<td>Maximum plasma concentration</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>C\textsubscript{trough}</td>
<td>Trough plasma concentration</td>
</tr>
<tr>
<td>C\textsubscript{trough}/dose</td>
<td>Dose-adjusted trough plasma concentration</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DADLE</td>
<td>[D-Ala\textsuperscript{2},D-Leu\textsuperscript{5}]-enkephalin</td>
</tr>
<tr>
<td>DAMGO</td>
<td>[D-Ala\textsuperscript{2},N-Me-Phe\textsuperscript{4},Gly\textsuperscript{5}-ol]-enkephalin</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>Deoxynucleoside triphosphate</td>
</tr>
<tr>
<td>DPDE</td>
<td>[D-Pen\textsuperscript{2,5}]-enkephalin</td>
</tr>
<tr>
<td>DPM</td>
<td>Disintegrations per minute</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>FCS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank’s buffered salt solution</td>
</tr>
<tr>
<td>HD</td>
<td>High dose</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IC\textsubscript{50}</td>
<td>50% inhibitory concentration</td>
</tr>
<tr>
<td>IDRS</td>
<td>Australian Illicit Drug Reporting System</td>
</tr>
<tr>
<td>IDU</td>
<td>Injecting drug users</td>
</tr>
<tr>
<td>kb</td>
<td>kilobases</td>
</tr>
<tr>
<td>LAAM</td>
<td>Levo-alpha-acetyl-methadol</td>
</tr>
<tr>
<td>LD</td>
<td>Linkage disequilibrium</td>
</tr>
<tr>
<td>M-6-G</td>
<td>Morphine-6-glucuronide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MEM</td>
<td>Minimal essential medium with Earl’s salts</td>
</tr>
<tr>
<td>MMT</td>
<td>Methadone maintenance treatment</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>NBD</td>
<td>Nucleotide binding domain</td>
</tr>
<tr>
<td>ND</td>
<td>Normal dose</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>$P_{app}$</td>
<td>Apparent permeability</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td>PCR - restriction fragment length polymorphism</td>
</tr>
<tr>
<td>P-gp</td>
<td>P-glycoprotein</td>
</tr>
<tr>
<td>PK/PD</td>
<td>Pharmacokinetic/pharmacodynamic</td>
</tr>
<tr>
<td>Pop-PK</td>
<td>Population-pharmacokinetic</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Quantitative real time - PCR</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulphate – polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>Half-life</td>
</tr>
<tr>
<td>TDM</td>
<td>Therapeutic drug monitoring</td>
</tr>
<tr>
<td>TEER</td>
<td>Transepithelial electrical resistance</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>Time to maximum plasma concentration</td>
</tr>
<tr>
<td>TMD</td>
<td>Transmembrane domain</td>
</tr>
<tr>
<td>V</td>
<td>Variant allele or digest fragment</td>
</tr>
<tr>
<td>$V_d$</td>
<td>Volume of distribution</td>
</tr>
<tr>
<td>Wt</td>
<td>Wild-type allele or digest fragment</td>
</tr>
</tbody>
</table>