The *in vitro* and *in vivo* Formation and Potency of 6β-Naltrexol, the Major Human Metabolite of Naltrexone

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Abstract

6β-Naltrexol is the major human metabolite of naltrexone, which is an opioid receptor antagonist used in the treatment of opioid and alcohol dependence. This metabolite is thought to contribute to the pharmacological effects of naltrexone, particularly the longer duration of naltrexone compared to naloxone (the prototypical opioid receptor antagonist), but to what extent has not been fully described.

6β-Naltrexol was synthesised from naltrexone in order to conduct the studies contained in this thesis as it was not commercially available at the time. Additionally, a validated HPLC assay method needed to be developed to quantify naltrexone and 6β-naltrexol for the *in vivo* and *in vitro* studies contained within. 6β-Naltrexol was successfully synthesised, and the HPLC assay was developed for simultaneous analysis of the parent and metabolite in a number of biological fluids, and performed with a high degree of precision and accuracy throughout.

The enzyme kinetics for the formation of 6β-naltrexol from naltrexone were determined *in vitro* in human liver cytosolic and microsomal preparations. Additionally, several compounds were tested for their likelihood of inhibition of this formation. The hepatic enzymatic formation of 6β-naltrexol from naltrexone was confined to the cytosolic and not the microsomal fraction, exhibited considerable intersubject variability and could be inhibited by a number of compounds. The most potent of these were certain steroid hormones, and naloxone.

The *in vivo* pharmacokinetics and bioavailability of naltrexone, and the formation of 6β-naltrexol, were also assessed after oral and intravenous administration of naltrexone to healthy volunteers. Naltrexone and 6β-naltrexol were quantified in the plasma, urine and saliva of these subjects. Additionally, the correlation between 6β-naltrexol concentrations and increased subjective side-effects reported previously was assessed. As with the *in vitro* studies, there was a high degree of interindividual variation of pharmacokinetic parameters. It was found that saliva is possibly a better alternative to plasma in assessing naltrexone status following the 50 mg dose used clinically. There was no correlation between high biofluid concentrations of 6β-naltrexol and an increase in subjective side effects after intravenous or oral naltrexone administration.

Potency studies and assessment of the duration of antagonistic activity of 6β-naltrexol were conducted *in vitro* in electrically-stimulated guinea pig ileum preparations (blocking the
morphine-induced twitch height) and in vivo in mice (reversing morphine-induced antinociception). The potencies were compared to the parent naltrexone, and naloxone. Naltrexone was more potent than naloxone in the guinea pig ileum preparation and interestingly, 6β-naltrexol was found to be 4.5-fold more potent than naloxone, and nearly three times more potent than naltrexone in this preparation. The high potency found in the in vitro study was not reflected in the in vivo mouse study, in which 6β-naltrexol showed only 1/185th the potency of naltrexone. Whereas the in vivo potency of 6β-naltrexol was much lower than that of naltrexone or naloxone, the duration of action was much longer.

The in vivo potency of 6β-naltrexol is lower than that of its parent compound naltrexone, but the longer duration of action, and the significantly higher plasma concentrations of this metabolite after an oral dose of naltrexone indicate that 6β-naltrexol will contribute significantly to the therapeutic effects of naltrexone.
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 to Susan Porter 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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Susan J Porter
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My thanks go also to the Royal Adelaide Hospital for providing a Dawes Postgraduate Scholarship, without which my studies would never have begun. Thanks also to staff at the Institute of Medical and Veterinary Science, specifically Howard Morris, Peter O’Loughlin, Kingsley Valledares, David Neilsen and Allan Rofe, for their continued financial support which enabled me to eat whilst doing this project.

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List of abbreviations used in this thesis

The abbreviations and prefixes of the International System of units have been used in this thesis, except for the alternatives listed below. Additional abbreviations and terminology, and pharmacokinetic symbols are also listed.

AKR aldo-keto reductase family of enzymes
ALT alanine aminotransferase
AST aspartate aminotransferase
AUC area under the concentration-time curve
cAMP cyclic 3',5'-adenosine monophosphate
CL total systemic clearance
CL\(_{\text{int}}\) intrinsic clearance
CL/F apparent oral clearance
CL\(_{\text{R}}\) apparent renal clearance
\(C_{\text{max}}\) maximum measured concentration
CNS central nervous system
COMT catechol –O-methyltransferase
CV coefficient of variation (expressed as a percentage)
CYP450 cytochrome P450 enzyme
DADLE D-Ala-\(\Delta\)-leu-enkephalin
DAMGO [D-Ala\(^2\),N-MePhe\(^4\),Gly-ol\(^5\)]enkephalin
DD dihydrodiol dehydrogenase
DSLET [D-Ser\(^2\),Leu\(^5\),Thr\(^6\)]enkephalin
DRG dorsal root ganglion
DSM-IV Diagnostic and Statistical Manual (Volume IV)
ECD electrochemical detection
EC\(_{50}\) effective concentration eliciting 50% of maximal effect
ED\(_{50}\) effective dose eliciting 50% of maximal effect
fu fraction unbound in plasma
GC gas chromatography
GPCR guanine nucleotide binding (G) –protein coupled receptor
HEK human embryonic kidney
HMM 2-hydroxy-3-methoxynaltrexone
HPLC high pressure (performance) liquid chromatography
HQC high quality control
IC\(_{50}\) concentration of antagonist that inhibits agonist action by 50%
ID\(_{50}\) dose of antagonist that inhibits agonist action by 50%
icv intracerebroventricular
im intramuscular
ip intraperitoneal
it intrathecal
IUPHAR International Union of Pharmacologists
IV intravenous
\(K_A\) equilibrium dissociation constant of a drug for its receptor
\(K_i\) inhibition constant
\(K_m\) affinity constant of enzyme for substrate, concentration at which reaction is half of \(V_{\text{max}}\) (Michaelis-Menten dissociation constant)
LOQ limit of quantification
LQC low quality control
M6G morphine-6-glucuronide
MQC medium quality control
MS mass spectrometry
MSC Methadone Symptoms Checklist
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>n</td>
<td>number within a sample</td>
</tr>
<tr>
<td>NADP(H)</td>
<td>nicotinamide adenine dinucleotide phosphate (reduced form)</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>pA₂</td>
<td>measure of affinity of an antagonist to its receptor (-log Kᵦ)</td>
</tr>
<tr>
<td>PFPA</td>
<td>pentafluoropropionic acid</td>
</tr>
<tr>
<td>PK</td>
<td>pharmacokinetic</td>
</tr>
<tr>
<td>pKa</td>
<td>acidity constant log₁₀ transformed (pH at which 50% of the compound is ionised)</td>
</tr>
<tr>
<td>POMS</td>
<td>Profile of Mood States</td>
</tr>
<tr>
<td>QC</td>
<td>quality control</td>
</tr>
<tr>
<td>r</td>
<td>correlation coefficient</td>
</tr>
<tr>
<td>r²</td>
<td>coefficient of determination</td>
</tr>
<tr>
<td>Rf</td>
<td>retention factor</td>
</tr>
<tr>
<td>RI</td>
<td>reference interval</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>sc</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>³⁵S-GTPγS</td>
<td>³⁵S-guanosine triphosphate-gamma S</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>half-life</td>
</tr>
<tr>
<td>tlc</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>Tmax</td>
<td>time at which maximum concentration is achieved</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>V</td>
<td>rate of formation of substrate</td>
</tr>
<tr>
<td>Vₐ</td>
<td>apparent volume of distribution</td>
</tr>
<tr>
<td>Vₘₐₓ</td>
<td>maximum reaction velocity</td>
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</tbody>
</table>