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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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_{int}	intrinsic clearance
CL/F	apparent oral clearance
CL_R	apparent renal clearance
C_{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-Δ-leu-enkephalin
DAMGO	[D-Ala^2,*N*-$MePhe^4$,$Gly\text{-}ol^5$]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
HMN	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_{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

MW	molecular weight
n	number within a sample
NADP(H)	nicotinamide adenine dinucleotide phosphate (reduced form)
NMR	nuclear magnetic resonance
pA_2	measure of affinity of an antagonist to its receptor (-log K_B)
PFPA	pentafluropropionic acid
PK	pharmacokinetic
pKa	acidity constant log_{10} transformed (pH at which 50% of the compound is ionised)
POMS	Profile of Mood States
QC	quality control
r	correlation coefficient
r^2	coefficient of determination
Rf	retention factor
RI	reference interval
rpm	revolutions per minute
sc	subcutaneous
SD	standard deviation
SEM	standard error of the mean
^{35}S-GTPγS	^{35}S-guanosine triphosphate-gamma S
SNP	single nucleotide polymorphism
$t_{1/2}$	half-life
tlc	thin layer chromatography
Tmax	time at which maximum concentration is achieved
UV	ultraviolet
V	rate of formation of substrate
V_d	apparent volume of distribution
V_{max}	maximum reaction velocity