PHYSICOCHEMICAL DETERMINANTS OF THE NON-SPECIFIC BINDING OF DRUGS TO HUMAN LIVER MICROSOMES

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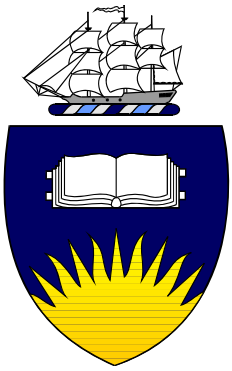

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DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

James A McLure

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PUBLICATIONS AND AWARDS

Publications

McLure JA, Sorich MJ, Miners JO, Smith PA, Birkett DJ 2002, 'Determinants of the membrane binding of drugs in microsomal *in vitro* systems', *Proceedings of the Australian Health and Medical Research Congress*, Poster Abstracts 2118.

McLure, JA, Birkett, DJ, Miners, JO 2004, 'Determinants of the non-specific binding of drugs to human liver microsomes', *Clinical and Experimental Pharmacology and Physiology* 31, PO-209.

McLure, James, Miners, John, Birkett, Donald 2006, 'The development of a fluorescence technique for measuring the non-specific binding of drugs to human liver microsomes' *Acta Pharmacologia Sinica* July; Supplement 1, p. 221, P170084.

McLure, JAL 2006, 'Development of an 8-anilinonapthalene-1-sulfonate (ANS) fluorescence technique for measuring the non-specific binding of drugs to human liver microsomes' *Proceedings of the Australian Health and Medical Research Congress* ISSN 1447-6610, p. 416, Abstract # 1364.

Awards

2000 - 2002 Private Scholarship, Department of Clinical Pharmacology, Flinders University, Bedford Park, South Australia

2003 Flinders University Research Scholarship, Flinders University, Bedford Park, South Australia

ABSTRACT

Accurate determination of the *in vitro* kinetic parameters K_m (Michaelis constant) and K_i (inhibition constant) is critical for the quantitative prediction of *in vivo* drug clearance and the magnitude of inhibitory drug interactions. A cause of inaccuracy *in vitro* arises from the assumption that all drug added to an incubation mixture is available for metabolism or inhibition. Many drugs bind non-specifically to the membrane of the *in vitro* enzyme source.

The aims of this thesis were to: 1) investigate the comparative importance of lipophilicity (as log P), and pK_a as determinants of the non-specific binding of drugs to human liver microsomes; 2) develop and validate an ANS fluorescence technique for measuring the non-specific binding of drugs to human liver microsomes; 3) characterise the non-specific binding of a large dataset of physicochemically diverse drugs using the ANS fluorescence procedure; 4) evaluate relationships between selected physicochemical characteristics and the extent of non-specific binding of drugs to human liver microsomes and; 5) computationally model the non-specific binding of drugs to discriminate between high binding ($f_{u(mic)} < 0.5$) and low binding ($f_{u(mic)} \geq 0.5$) drugs.

The comparative binding of the basic drugs atenolol (log P = 0.1; $f_{u(mic)} = 1.00$), of propranolol (log P = 3.1; $f_{u(mic)} = 0.36 - 0.84$), and imipramine (log P = 4.8; $f_{u(mic)} = 0.42 - 0.82$) suggested that lipophilicity is a major determinant of non-specific binding. In contrast, the comparative binding of diazepam ($pK_a = 3.3$; $f_{u(mic)} = 0.69 - 0.80$), a neutral compound; and the bases propranolol ($pK_a = 9.5$; $f_{u(mic)} = 0.36 - 0.84$) and lignocaine ($pK_a = 9.5$; $f_{u(mic)} = 0.98$), indicated that pK_a was not a determinant of the extent of non-specific binding. The non-binding of lignocaine, a relatively

lipophilic base, was unexpected and confirmed by the non-binding of the structurally related compounds bupivacaine and ropivacaine. These results implicated physicochemical characteristics other than lipophilicity and charge as important for the non-specific binding of drugs to human liver microsomes.

An assay based on 1-anilinonaphthalene-8-sulfonate (ANS) fluorescence was developed using the seven drugs employed in the initial study. Non-specific binding data from equilibrium dialysis and the ANS fluorescence methods were compared and a linear correlation ($r^2 = 0.92$, $p < 0.01$) was observed at drug concentrations of 100 and 200 μM. The approach was further validated by characterising the microsomal binding of nine compounds (bupropion, chloroquine, chlorpromazine, diflunisal, flufenamic acid, meclofenamic acid, mianserine, trifluprom azine, and verapamil) using both binding methods (i.e. equilibrium dialysis and ANS fluorescence). A significant logarithmic relationship ($r^2 \geq 0.90$) was demonstrated between $f_{u(mic)}$ and the modulus of ANS fluorescence for all drugs and for basic drugs alone at concentrations of 100 and 200 μM, while the acidic/neutral drugs showed a significant linear relationship ($r^2 \geq 0.84$) at these two concentrations ($p < 0.01$). The non–binding of bupropion provided further evidence that physicochemical properties other than log P and charge were important for non-specific binding of drugs to human liver microsomes.

The ANS fluorescence technique was then used to characterise the non-specific binding of 88 physicochemically diverse compounds. In general, acids and neutrals bound to a 'low' extent ($f_{u(mic)} \geq 0.5$) whereas bases bound the full $f_{u(mic)}$ range (0.0001 – 1). Statistically significant relationships were observed between the non-specific binding of bases and log P, the number of hydrogen bond donors and hydrogen bond acceptors per molecule, and molecular mass.

Preliminary *in silico* modeling of the dataset generated by the ANS fluorescence technique, using the program ROCS, provided discrimination of all but one (itraconazole) of the 'high' binding bases. However, there were 14 false positives, resulting in low overall prediction accuracy.

Taken together, the studies conducted in this thesis provide important insights into the physicochemical factors that determine the non-specific binding of drugs to human liver microsomes.

ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, Excretion
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicology
ANS	1-anilino-8-naphthalene sulfonate
B_{max}	maximum binding capacity
Caco-2	intestinal cell line from human colorectal cancer
Chol	cholesterol
CL	clearance
C_B	concentration of bound drug
C_F	concentration of free drug
CL_H	hepatic clearance
CL_{int}	intrinsic clearance of drug metabolising enzyme(s)
C_0	initial concentration at time zero
CL_S	systemic clearance
C_{SS}	concentration of drug in plasma at steady state
C_t	drug concentration at time t after the dose
CYP	cytochrome P450
D	drug
D_F	free drug
DMSO	dimethylsulfoxide
D_N	dispersion number
E_H	hepatic extraction ratio
f_m	fraction of dose metabolised along pathway of interest
f_u	fraction of drug unbound in blood
$f_{u(inc)}$	fraction of drug unbound in an incubation

f_{um}	fraction of drug unbound in an incubation medium
$f_{u(mic)}$	fraction of drug unbound in microsomes
$f_{u(T)}$	fraction of drug unbound in tissue
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HPLC	high performance liquid chromatography
I	inhibitor
I_u	unbound inhibitor
IND	Investigational New Drug
k	elimination rate constant
K_D	dissociation constant
K_i	inhibition constant
K_m	Michaelis constant (substrate concentration at half maximal velocity)
$K_{m(app)}$	apparent Michaelis constant
log P	log of the concentration of drug in the lipid phase / concentration of drug in the aqueous phase
M	metabolised
MM	molecular mass
MW	molecular weight
NDA	New Drug Application
NMR	nuclear magnetic resonance
NNN'N' TMED	NNN'N' tetramethylethylenediamine
NSAID	non steroidal anti-inflammatory drug
PB	potassium phosphate buffer, 0.1M, pH 7.4

PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PS	phosphatidylserine
PSA	polar surface area
Q_H	liver blood flow
QSAR	Quantitative Structure-Activity Relationship
R_c	ratio of the areas under the plasma drug concentration time curves in the presence and absence of inhibitor
ROCS	Rapid Overlay of Chemical Structures
S	substrate
SD	standard deviation
SM	sphingomyelin
t	time
$t_{1/2}$	half life of a drug dose
UGT	UDP-glucuronosyltransferase
V, v	velocity or rate of metabolite formation
V_d	volume of distribution
V_{max}	maximal velocity of a reaction at a saturating substrate concentration
V_P	plasma volume
V_T	tissue volume