CHEMOPREVENTION STUDIES WITH PACIFIC FOODSTUFFS: EFFECTS ON XENOBIOTIC METABOLISING ENZYMES AND CYTOTOXICITY.

By

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Abstract

The aims of this thesis were to examine:(1) the effects of acute and chronic dietary exposure of the pro-carcinogen, IQ on the expression and activity of xenobiotic metabolising enzymes; (2) the ability of foodstuffs chronically (individually and in combination with the dietary pro-carcinogen IQ) to alter the xenobiotic metabolising enzymes; (3) whether tissue-specific effects of the foodstuffs on XME could explain pathological results; and (4) whether any isolated fraction of the foodstuffs acted directly as a toxicant or stimulated cell growth *in vitro*.

For the chronic study, male Fischer 344 rats were fed defined AIN-76A diet ± 10 % foodstuff (taro, kumara, pineapple, or coconut), with or without IQ (300ppm for 52 weeks). In the acute study, male Fischer 344 rats were fed defined AIN-76A diet and gavaged with IQ (20 $mg.kg^{-1}$, 3 days). Pathology was observed and changes in enzyme activities and expression examined. Simple fractionation of the foodstuffs was also performed and their effects on cell viability and enzyme expression were investigated *in vitro* in freshly isolated rat hepatocytes and immortal human HT29 and P388 cells.

In the liver, acute exposure to IQ affected enzymes consistent with complex xenobiotic response element / glucocorticoid response element activation, whereas chronic exposure was consistent with activation of the antioxidant response element. No pattern of enzyme change was obvious in other organs. Inclusion of South Pacific foodstuffs in the diet at 10 % had a marked effect on enzyme activity in the liver and lung, although there was no consistent pattern for enzyme changes for a particular enzyme or foodstuff. Enzyme activity in the colon was below the level of detection. There was a complex response when IQ was included with the foodstuffs, which did not resemble the response to either IQ or the foodstuff alone. Fractionantion of foodstuffs and *in vitro* cell-based work did not reveal the presence of compounds in the foodstuffs that explained the complex changes in pathology or XME observed *in vivo*.

The interaction between foodstuffs and IQ in the body was complicated. This may be explained by the presence of many compounds within the foodstuffs that have additive, synergistic, or inhibitory actions on many pathways in the body and underlines the complexity of using whole foodstuffs, rather than a single, purified constituent, in chemoprevention studies.

Publications Arising From This Thesis

McPherson, R. A. C., L. R. Ferguson, et al. (2000). Effect of kumara (*Ipomoea batatis*) and chronic dietary exposure to 2-amino-3-methyl-imidazo[4,5-*f*]quinoline on xenobiotic metabolising enzymes in the male Fischer 344 rat. The Annual meeting of Society for Free Radical Research (SFRR) Australasia, Christchurch.

McPherson, R. A. C., M. D. Tingle, et al. (1999). "Effect of chronic dietary exposure to IQ on glutathione *S*-transferase in the male Fischer 344 rat liver." Proceedings Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists **6**: 181.

McPherson, R. A. C., M. D. Tingle, et al. (1999). "Effect of dietary exposure to IQ on phase I and II XME in the male Fischer rat liver." Proceedings of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists **6**: 30.

McPherson, R. A. C., M. D. Tingle, et al. (2000). "Dietary intervention studies in the rat: Organ-specific effects on the modulation of xenobiotic metabolising enzymes by IQ." Proceedings Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists **8**: 114.

McPherson, R. A. C., M. D. Tingle, et al. (2000). "Effect of pacific island foodstuffs on the enzyme modulation resulting from chronic dietary exposure to 2-amino 3-methylimidazo [4,5-*f*]quinoline (IQ)." Proceedings Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists **7**: 114.

McPherson, R. A. C., M. D. Tingle, et al. (2001). "Contrasting effects of acute and chronic dietary exposure to 2-amino-3-methyl-imidazo[4,5-*f*]quinoline (IQ) on xenobiotic metabolising enzymes in the male Fischer 344 rat: implications for chemoprevention studies." European Journal of Nutrition **40**(1): 39-47.

McPherson, R. A. C., M. D. Tingle, et al. (2001). "Effects of the dietary carcinogen IQ and Pacific island foods on XME in male Fischer 344 rat lung and liver." Toxicology **164**(1-3): 147.

Dedication

This thesis is dedicated in loving memory of Andrew James Rea
(13^{th} September 1976 – 2^{nd} February 2001).

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Abbreviations

2D	2-Dimensional
4-Ipomeanol	1-(3-Furyl)-4-hydroxypentanone
4-Mu	4-Methyl umbelliferone
4-MuG	4-Methyl umbelliferone glucuronide
$4NO_2BCl$	4-Nitrobenzylchloride
ADME	Absorption, Distribution, Metabolism and Elimination
AFB_1	Aflatoxin B_1
Ah-receptor	Aryl-hydrocarbon receptor
AhRR	Ah-receptor repressor
AIN-76A	American Institute of Nutrition diet number 76A
AIN-93G	American Institute of Nutrition diet number 93G
ALDH-3	Aldehyde dehydrogenase 3
AP-1	Activator Protein-1
ARE	Antioxidant Response Element
ARNT	Aryl-hydrocarbon receptor nuclear translocator
BaP	Benzo[*a*]pyrene
BCA	Bicinchoninic acid
BSA	Bovine Serum Albumin
CAR	Constitutive Androsterone Receptor
CDNB	1-Chloro 2,4-dinitrobenzene
CHAPS	3-[(3-Cholamidopropyl) dimethylammonio]-1-propane sulphonate
CYP1A	Cytochrome P450 1A
CYP1B	Cytochrome P450 1B
CYP2B	Cytochrome P450 2B
CYP2E	Cytochrome P450 2E
CYP3A	Cytochrome P450 3A
CYP450	Cytochrome P450
DCNB	3-4-Dichloro nitrobenzene
DCPIP	Dichloroindophenol
DMSO	Dimethylsulfoxide
DRE	Dioxin Response Element
DTT	Dithiothreitol
ECL	Enhanced chemiluminescence
EpRE	Electrophile responsive element

EtOH	Ethanol
F344	Fischer 344
FCS	Fetal Calf Serum
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
GST	Glutathione *S*-transferase
GST-A	Glutathione *S*-transferase alpha
GST-M	Glutathione *S*-transferase mu
GST-T	Glutathione *S*-transferase theta
HCA	Heterocyclic amine
HEPES	4-(2-Hydroxyethyl)piperazine-1-(2-ethanesulfonic acid)
HSP90	Heat shock protein, number 90
IARC	International Agency for Research on Cancer
IEF	Isoelectric Focusing
IPG	Immobilized pH gradient
IQ	2-Amino-3-methylimidazo- [4,5-*f*] quinoline
MAP kinase	Mitogen-activated protein kinase
MeIQX	2-Amino-3, 4-dimethylimidazo- [4,5-*f*] quinoline
MEM	Minimum Essential Medium
MeOH	Methanol
MDR	Multidrug resistance gene
MRP	Multidrug resistance protein
NAD(P)H	Nicotinamide adenine dinucleotide phosphate
NADH	Nicotinamide adenine dinucleotide
NHR	Nuclear Hormone Receptor
NNK	4-(methylnitroso)-1-(3-pyridyl)-1-butanone
NQO1	NAD[P]H:quinine acceptor oxidoreductase
Nrf	Nuclear factor, erythroid-derived 2-like (transcription factor)
PAR	Pregnane activated receptor
PBREMα	Phenobarbitone Response Element Modulator-α
PCB	Polychlorinated biphenyls
PGHS	Prostaglandin-H synthase
PhIP	2-Amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine
pi	Isoelectric point
pNP	*p*-Nitrophenol phosphate

PPAR-α	Peroxisome Proliferator Activated Receptor-α
ppm	Parts per million
PVDF	Polyvinylidene difluoride
PXR-RE	Pregnane X receptor response element
PXR	Pregnane X Receptor
RMIT	Royal Melbourne Institute of Technology
ROS	Reactive oxygen species
s.e.m	Standard error of the mean
SDS	Sodium dodecyl sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SXR	Steroid X receptor
TLC	Thin layer chromatography
UDPGA	Uridine di-phosphate glucuronic acid
UGT	Uridine di-phosphate-glucuronosyl transferase
XME	Xenobiotic metabolising enzyme
XRE	Xenobiotic response element