Effect of Herbal Medicines on the Pharmacokinetics and Pharmacodynamics of Warfarin in Healthy Subjects

By

Xuemin Jiang

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Preface

The work described in this thesis was carried out by the author in the Faculty of Pharmacy, The University of Sydney, under the supervision of Dr Andrew J McLachlan and Dr Colin C Duke. This thesis has not been submitted for a degree at any other university. Full acknowledgement has been made where the work of others has been cited or used. A list of publications is included in support of this thesis.

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Publications in Support of this Thesis

- Jiang X, Williams KM, Liauw W, Ammit AJ, Roufogalis BD, Duke CC, Day RO, McLachlan AJ, Effect of St John's wort and ginseng on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects, *British Journal of Clinical Pharmacology* 2004;57:592-9.

- Jiang X, Williams KM, Liauw W, Ammit AJ, Roufogalis BD, Duke CC, Day RO, McLachlan AJ, Effect of ginkgo and ginger on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects, *British Journal of Clinical Pharmacology* (Under revision).

Papers Presented at Scientific Meeting

- Jiang X, Williams KM, Liauw W, Ammit AJ, Roufogalis BD, Duke CC, Day RO, McLachlan AJ, Pharmacokinetic-pharmacodynamic modelling to investigate herb-drug interactions with warfarin. 8th World Congress on Clinical Pharmacology & Therapeutics (2004). Poster

- Jiang X, Williams KM, Liauw W, Ammit AJ, Roufogalis BD, Duke CC, Day RO, McLachlan AJ, Effect of ginkgo and ginger on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects. Australasian Pharmaceutical Science Association **(APSA)** Annual Conference, Sydney (2003). Podium.

- Jiang X**,** Williams KM, Liauw W, Ammit AJ, Roufogalis BD, Duke CC, Day RO, McLachlan AJ, Effect of St John's wort and ginseng on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects. American Association of Pharmaceutical Scientist **(AAPS)** Annual Conference and Exposition – Salt Lake City, 26-30, October, 2003. Poster.

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Abbreviations

ADP	Adenosine disphosphate
ANOVA	Analysis of variance
APTT	Activated partial prothrombin time
ATP	Adenosine triphosphate
AUC	Area under the plasma drug concentration-time curve
BP	British Pharmacopoeia
cAMP	Adenosine 3',5'-cyclic phosphate
cGMP	cyclic guanosine monophosphate
CI	Confidence interval
C_{max}	Maximum drug concentration observed in plasma
C_{min}	Minimum drug concentration observed in plasma
C_R	R-warfarin concentration
C_S	S-warfarin concentration
$C_{50,R}$	Concentration of R-warfarin that produces 50% inhibition of PCA
$C_{50,S}$	Concentration of S-warfarin that produces 50% inhibition of PCA
CL	Total clearance of drug from plasma
CLu	Clearance of unbound drug
CNS	Central nervous system
CV	Coefficient of variation
CYP	Cytochrome P450
DA	Dopamine
cDNA	Complementary deoxyribonucleic acid
E_{max}	Maximum effect
Em	Emission
Ex	Excitation
F	Bioavailability of drug or herbal constituent
FDA	Food and Drug Administration
fe	Fraction of drug or herbal constituent excreted unchanged in urine
fu	Fraction of unbound drug concentration in plasma
GC	Gas chromatography
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GS	Ginseng
h	Hour
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
IC_{50}	The concentration that produces 50% inhibition of PCA
INR	International normalised ratio of prothrombin time
I.S.	Internal standard
k	Elimination rate constant
ka	Absorption rate constant
k_d	Degradation rate constant of the prothrombin complex
LCMSMS	Liquid chromatography mass spectrometry/mass spectrometry
LOQ	Limit of quantitation
mRNA	Messenger ribonucleic acid

min	Minute
MW	Molecule weight
NA	Noradrenaline
NSAIDs	Non steroidal anti-inflammatory drugs
QC	Quality control
PAF	Platelet activating factor
PCA	Prothrombin complex activity
PK/PD	Pharmacokinetics/Pharmacodynamics
PT	Prothrombin time
PXR	Pregnane X receptor
r^2	Coefficient of determination
R_{deg}	Degradation rate of prothrombin complex
R_{syn}	Synthesis rate of prothrombin complex
SD	Standard deviation
sec	Second
SJW	St John's wort
$t_{1/2}$	Half-life
t_d	Time between the start of absorption of the drug and the initiation of anticoagulant
TGA	Therapeutic Goods Administration
TXA_2	Thromboxane A_2
TLC	Thin-layer Chromatography
t_{max}	Time at which the highest drug concentration occurs
UV	Ultraviolet
USP	United States Pharmacopoeia
UER	Urinary excretion rate
V	Volume of distribution
WF	Warfarin
γ	Slope factor in concentration-response relationship

Abstract

Herbal medicines are widely used in our community. A survey of Australian consumers indicated that 60% had used complementary and/or alternative medicines in the past year with the majority not informing their doctor that they were using herbal medicines. Little is known about the potentially serious consequences of interactions between herbal and conventional medicines. Warfarin has an important role in treating people with heart disease, yet it has a narrow therapeutic range, is highly bound to plasma proteins, and is metabolised by cytochrome P450. This creates the potential for life-threatening interactions with other drugs and foods leading to excessive bleeding. Hence, warfarin is one of the most frequently investigated drugs for interaction studies. Early clinical reports suggest that there exists the potential for an interaction between warfarin and four herbal medicines: St John's wort, ginseng, ginkgo and ginger. However, these herb-drug combinations have never been conclusively studied.

The two clinical studies conducted as part of this research had an identical study design. Twenty-four healthy male subjects were recruited into the two separate studies. This was an open label, three-way crossover randomised study in twelve healthy male subjects, who received a single 25 mg dose of warfarin alone or after 14 days pre-treatment with St John's wort, or 7 days pre-treatment with ginseng. Dosing with St John's wort or ginseng was continued for 7 days after administration of the warfarin dose in study I or who received a single 25 mg dose of warfarin alone or

after 7 days pre-treatment with recommended doses of ginkgo or ginger from single ingredient products of known quality. Dosing with ginkgo or ginger was continued for 7 days after administration of the warfarin dose in study II. Platelet aggregation, international normalised ratio (INR) of prothrombin time, warfarin enantiomer protein binding, warfarin enantiomer concentrations in plasma and S-7-hydroxywarfarin concentration in urine were measured in both studies. Statistical comparisons were made using ANOVA and 95% confidence interval (CI) for mean value and 90% CI for geometric mean ratio value are reported.

In study I, the mean (95% CI) apparent clearance of S-warfarin after warfarin alone or with St John's wort or ginseng were, respectively, 198 (174 – 223) ml/h, 269 (241 – 297) ml/h and 220 (201 – 238) ml/h. The respective apparent clearances of R-warfarin were 110 (94 – 126) ml/h, 142 (123 – 161) ml/h and 119 (106 – 131) ml/h. The mean ratio of apparent clearance for S-warfarin was 1.29 (1.16-1.46) and for R-warfarin was 1.23 (1.11-1.37) when St John's wort was co-administered. The mean ratio of AUC_{0-168} of INR was 0.79 (0.70 - 0.95) when St John's wort was co-administered. The urinary excretion ratio of S-7-hydroxywarfarin after administration of warfarin alone was 0.04 (0.03 – 0.06) mg/h and there was no significant difference following treatment with either St John's wort 0.03 (0.02 – 0.04) mg/h or ginseng 0.03 (0.02 – 0.04) mg/h. The ratio of geometric means for S-7-hydroxywarfarin UER was 0.82 (0.61-1.12) for St John's wort, and 0.68 (0.50-0.91) for ginseng. St John's wort and ginseng did not affect the apparent volumes of distribution or protein binding of warfarin enantiomers.

In study II, the mean (95% CI) apparent clearance of S-warfarin after warfarin alone, with ginkgo or ginger were 189 (167 – 210) ml/h, 200 (173 – 227) ml/h and 201 (171 – 231) ml/h, respectively. The respective apparent clearances of R-warfarin were 127 (106 – 149) ml/h, 126 (111 – 141) ml/h and 131 (106 – 156) ml/h. The mean ratio of apparent clearance for S-warfarin was 1.05 (0.98 -1.12) and for R-warfarin was 1.00 (0.93 -1.08) when co-administered with ginkgo. The mean ratio of AUC_{0-168} of INR was 0.93 (0.81 -1.05) when co-administered with ginkgo. The mean ratio of apparent clearance for S-warfarin was 1.05 (0.97 -1.13) and for R-warfarin was 1.02 (0.95 - 1.10) when co-administered with ginger. The mean ratio of AUC_{0-168} of INR was 1.01 (0.93 -1.15) when co-administered with ginger. The urinary excretion ratio (UER) of S-7-hydroxywarfarin after administration of warfarin alone was 0.04 (0.03 – 0.05) mg/h and there was no significant difference following treatment with either ginkgo 0.04 (0.03 – 0.04) mg/h or ginger 0.03 (0.02 – 0.04) mg/h. The ratio of geometric means for S-7-hydroxywarfarin UER was 1.07 (0.69-1.67) for ginkgo, and 1.00 (0.64-1.56) for ginger. Ginkgo and ginger did not affect the apparent volumes of distribution or protein binding of either S-warfarin or R-warfarin.

In conclusion, St John's wort significantly induced the apparent clearance of both S-warfarin and R-warfarin, which in turn resulted in a significant reduction in the pharmacological effect of *rac*-warfarin. Ginseng, ginkgo and ginger at recommended doses affect neither clotting status, nor the pharmacokinetics or pharmacodynamics of either S-warfarin or R-warfarin in healthy subjects.