Enantioselectivity in Clinical Pharmacology: Theoretical Considerations and Studies with Ibuprofen

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Science of the University of Adelaide.

Allan Mark Evans, B. Pharm.

Department of Clinical and Experimental Pharmacology,
University of Adelaide.

June 1989

Abstract

1. Ibuprofen is a chiral drug which is used clinically as a racemic mixture of its pharmacologically active (S(+)-ibuprofen; S-I) and inactive (R(-)-ibuprofen; R-I) enantiomers.

2. By theoretical analysis of plasma concentration-time profiles, generated for a model chiral drug, it was shown that pharmacokinetic and concentration-effect studies on racemic drugs, such as ibuprofen, can not be relied upon to produce accurate results unless the individual enantiomers are measured in the reference biological fluids.

3. To examine various aspects of the clinical pharmacology of ibuprofen, methods for quantifying total and unbound R-I and S-I in plasma, and ibuprofen and its metabolites in urine, were developed.

4. After oral administration of a range of doses of RS-I (200 to 1200mg) to healthy volunteers, ibuprofen absorption (as assessed by urinary recovery of ibuprofen and its metabolites) was extensive, and dose-independent. There was, for each enantiomer, a linear relationship between the area under the plasma unbound concentration-time curve (AUC_u) and dose, which indicated that the intrinsic clearance of each enantiomer was dose-independent. Each enantiomer exhibited concentration-dependent plasma protein binding which led to a non-linear relationship between the area under the total plasma concentration-time curve (AUC) and dose.

5. The intensity and duration of ibuprofens anti-platelet effects (assessed by measuring thromboxane generation during blood clotting) were dose-dependent. There was a close relationship between the concentration of plasma unbound S-I, and the degree of inhibition of cyclo-oxygenase, according to a sigmoidal concentration-effect model.

6. In healthy volunteers, cimetidine had no effect on the extent of absorption of ibuprofen or the total and unbound plasma concentration-time profiles for each enantiomer. It was concluded that cimetidine had no effect on the metabolism of ibuprofen, providing further evidence to support the concept that cimetidine has variable inhibitory effects on the various isozymes of cytochrome P-450.

7. Both enantiomers of ibuprofen were extensively (>99%) bound to plasma proteins. R-I was more extensively bound, such that the plasma unbound fraction of S-I exceeded that of R-I, by an average of 50 to 70%. Controlled *in vitro* studies indicated that the presence of one ibuprofen enantiomer could decrease the plasma protein binding of its mirror-image form.

Chapter 1
Introduction: Enantioselectivity in Clinical Pharmacology 1
1.1 Introduction to Optical Isomerism 2
1.1.1 Historical Aspects 2
1.1.2 Chirality, Enantiomers and Diastereomers 3
1.1.3 Nomenclature 6
1.1.4 Summary 6
1.2 Optical Activity and Clinical Pharmacology 7
1.2.1 Enantioselective Pharmacological Activity 8
1.2.2 Enantioselective Pharmacokinetics 10
1.2.2.1 Absorption 11
1.2.2.2 Distributional Processes 12
1.2.2.3 Metabolism 13
1.2.2.4 Renal and Biliary Excretion 16
1.2.2.5 The Clinical Implications of Enantioselective Pharmacokinetics 17
1.2.3 Comment 18
1.3 Measurement of Enantiomers in Biological Fluids 19

Chapter 2
The Consequences of Neglecting Enantioselectivity in Studies on Racemic Drugs: Some Theoretical Considerations 22
2.1 Introduction 22
2.2 Methods 22
2.2.1 Pharmacokinetic Properties of the Model Chiral Drug 22
2.2.2 Dosing 23
2.2.3 Simulations 24
2.3 Results and Discussion 25
2.3.1 Single Dose 25
2.3.2 Chronic Dose 32
2.4 Conclusion 37

Chapter 3
Ibuprofen: A Review of the Literature 39
3.1 Physico-chemical Properties of Ibuprofen 39
3.2 The Pharmacology of Ibuprofen 39
3.2.1 Historical Development 39

3.2.2 Pharmacological Properties 40
3.2.3 Toxicity 41
3.2.4 Mechanism of Action 42
3.3 Therapeutic Indications of Ibuprofen 46
3.4 Adverse Effects 47
3.5 The Metabolism of Ibuprofen 48
3.5.1 The Chiral Inversion of Ibuprofen and Other 2-PPA Derivatives 51
3.5.2 Incorporation of Ibuprofen into Triglycerides 56
3.5.3 Summary 57
3.6 The Pharmacokinetics of Ibuprofen 59
3.6.1 Gastrointestinal Absorption 59
3.6.2 Distributional Processes 61
3.6.3 Clearance 64
3.6.4 Extent of Inversion of R(-)-Ibuprofen 66
3.6.5 Terminal Half-Life 67
3.6.6 Factors Affecting the Pharmacokinetics of Ibuprofen 67
3.6.7 Comment 71

Chapter 4
Analysis of Ibuprofen Enantiomers in Human Plasma and of Ibuprofen and its Major Metabolites in Human Urine 72
4.1 Analysis of Total R(-)- and S(+)-Ibuprofen in Human Plasma 72
4.1.1 Introduction 72
4.1.2 Materials and HPLC Equipment 74
4.1.3 Methods 75
4.1.4 Performance of the Method 77
4.1.5 Attempts to Develop Alternative Methods 87
4.2 Analysis of Ibuprofen and its Metabolites in Urine 90
4.2.1 Introduction 90
4.2.2 Methods 91
4.2.3 Assay Development 92
4.3 Verification of Ibuprofen Content in BRUFEN® 200mg and 400mg Tablets 99

Chapter 5
Methodology for Measuring the Plasma
Protein Binding of Ibuprofen Enantiomers 100
5.1 Introduction 100
5.2 Methods 100
5.3 Method Development and Performance of the Method 104

Chapter 6
The Relationship Between the Pharmacokinetics of
Ibuprofen Enantiomers and the Dose of Racemic Ibuprofen
in Humans 114
6.1 Introduction 114
6.2 Methods 116
6.2.1 Subjects and Study Design 116
6.2.2 Drug Analyses in Biological Fluids 118
6.2.3 Pharmacokinetic Analysis 118
6.2.4 Statistical Analysis 119
6.3 Results 120
6.4 Discussion 138
6.5 Conclusion 145

Chapter 7
The Effect of Ibuprofen Dose on the Magnitude and
Duration of Platelet Cyclo-oxygenase Inhibition 146
7.1 Introduction 146
7.2 Methods 147
7.2.1 Subjects and Study Design 147
7.2.2 Assessment of Platelet Cyclo-oxygenase Inhibition 147
7.2.3 *In Vitro* Determination of the Influence of
R(-)-Ibuprofen, S(+)-Ibuprofen and RS-Ibuprofen
on TXB2 Generation During Whole Blood Clotting 150
7.2.4 Statistical Analysis 150
7.3 Results 153
7.4 Discussion 167
7.5 Conclusion 171

Chapter 8
The Effect of Cimetidine on the Pharmacokinetics of Ibuprofen Enantiomers 172
8.1 Introduction 172
8.2 Methods 174
8.2.1 Subjects and Study Design 174
8.2.2 Drug Analyses in Biological Fluids 176
8.2.3 Pharmacokinetic Analysis 176
8.2.4 Effect of Cimetidine on Ibuprofen Plasma Protein Binding *In Vitro* 177
8.2.5 Statistical Analysis 177
8.3 Results 178
8.4 Discussion 190
8.5 Conclusion 194

Chapter 9
The Plasma Protein Binding of Ibuprofen Enantiomers 195
9.1 Introduction 195
9.2 Methods 195
9.2.1 The Plasma Protein Binding of R(-)-Ibuprofen and S(+)-Ibuprofen in Humans After Oral Administration of Racemic Ibuprofen 195
9.2.2 *In Vitro* Binding of R(-)-Ibuprofen and S(+)-Ibuprofen in Plasma Obtained From Drug-Free Humans 195
9.3 Results 196
9.3.1 The Plasma Protein Binding of R(-)-Ibuprofen and S(+)-Ibuprofen in Humans After Oral Administration of Racemic Ibuprofen 196
9.3.2 *In Vitro* Binding of R(-)-Ibuprofen and S(+)-Ibuprofen in Plasma Obtained From Drug-Free Humans 197
9.4 Discussion 202
9.5 Conclusion 208

Chapter 10
General Conclusion 209

Appendices 213
Bibliography 232