THE EXPLORATORY CLINICAL DEVELOPMENT OF TUCARESOL, AN ANTISICKLING AGENT, USING A NOVEL SURROGATE MARKER.

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by

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CONTENTS

ABSTRACT .............................................................................. x

DECLARATION .......................................................................... xiv

DEDICATION .............................................................................. xvi

ACKNOWLEDGEMENTS .............................................................. xvii

GLOSSARY ................................................................................ xix

CHAPTER 1
INTRODUCTION .......................................................................... 1
  1.1 Introduction ...................................................................... 2
    1.1.1 The role of the clinical pharmacologist in exploratory drug development ................................. 2
  1.2 Sickle Cell Anemia ............................................................ 8
  1.3 A review of potential antisickling therapies ..................... 10
    1.3.1 Geratin inhibitors ..................................................... 11
    1.3.2 Cell-sickling inhibitors .......................................... 23
    1.3.3 Inhibitors of microvascular entrapment .................. 24
    1.3.4 Other treatments ............................................... 26
  1.4 Discussion ...................................................................... 28
CHAPTER 2

THE PHARMACY, PRECLINICAL PHARMACOLOGY, PHARMACOKINETICS AND TOXICOLOGY OF TUCARESOL

2.1 Introduction ......................................................... 30
2.2 Physical properties of tucaresol ................................... 31
2.3 Biochemistry: interaction with hemoglobin ...................... 31
2.4 Pharmacology ....................................................... 32
   2.4.1 In vivo / ex vivo ................................................. 32
   2.4.2 Whole animal .................................................. 33
2.5 Toxicology .......................................................... 34
   2.5.1 Acute i.v ......................................................... 34
   2.5.2 Acute oral ....................................................... 35
   2.5.3 Subacute oral ................................................... 36
   2.5.4 One month ...................................................... 37
   2.5.5 Teratogenicity .................................................. 38
   2.5.6 Mutagenicity .................................................. 39
2.6 Pharmacokinetics .................................................... 40
   2.6.1 Rat .............................................................. 40
   2.6.2 Rabbit .......................................................... 41
   2.6.3 Dog ............................................................. 42
   2.6.4 Monkey ........................................................ 43
2.7 Conclusions ......................................................... 44
CHAPTER 3
THE CLINICAL PHARMACOLOGY OF VALERESOL

3.1 Introduction ............................................. 64
3.2 Summary of preclinical data of valeresol ..................... 65
  3.2.1 Pharmacology ........................................... 65
  3.2.2 Toxicology ............................................. 65
  3.2.3 Pharmacokinetics ...................................... 66
3.3 Human studies with valeresol ................................. 66
  3.3.1 First human study ...................................... 66
  3.3.2 Study in sickle cell disease patients .................. 67
  3.3.3 Effects on moderate graded exercise in healthy
        volunteers ............................................... 68
  3.3.4 Effects on anaerobic threshold in healthy volunteers . 71
3.4 Conclusions ............................................... 74

CHAPTER 4
THE PHARMACOKINETICS, PHARMACODYNAMICS AND TOLERABILITY
OF SINGLE ORAL DOSES OF TUCARESOL IN HEALTHY MALE
VOLUNTEERS ................................................. 75

4.1 Introduction and Objectives ................................ 76
4.2 Study Design ............................................. 76
  4.2.1 General ................................................ 77
  4.2.2 Design Considerations ................................ 77
4.3 Subjects, Protocol and Methods ............................... 80
CHAPTER 5
THE EFFECTS OF A TITRATED LOADING DOSE OF TUCARESOL IN
HEALTHY MALE VOLUNTEERS
5.3 Study design ................................. 106
  5.3.1 General ................................. 106
  5.3.2 Design considerations ................. 108
  5.3.3 Statistical considerations .......... 110
  5.3.4 Exercise tests .......................... 110
  5.3.5 Psychometric tests ..................... 110
  5.3.6 Safety considerations ................. 116
5.4 Subjects, Protocol and Methods .............. 110
  5.4.1 Subjects ............................... 111
  5.4.2 Protocol ............................... 111
  5.4.3 Study drug ............................. 111
  5.4.4 Drug administration and dosages .... 112
  5.4.5 Schedule ............................... 113
  5.4.6 Laboratory Methods .................... 114
  5.4.7 Clinical measurements and procedures 114
  5.4.8 Data analysis .......................... 118
5.5 Results .................................... 120
  5.5.1 Subjects ................................ 120
  5.5.2 Protocol compliance and modifications 120
  5.5.3 Doses administered ...................... 120
  5.5.4 Pharmacokinetics ....................... 121
  5.5.5 Haemoglobin modification - %MOD ..... 121
  5.5.6 Cardiovascular and psychometric data 122
  5.5.7 Adverse experiences .................... 122
CHAPTER 6

THE PHARMACOKINETICS, TOLERABILITY AND EFFECTS ON
HAEMOLYSIS OF MULTIPLE DOSES OF TUCARESOL IN PATIENTS WITH
SICKLE CELL DISEASE .................................................. 169

6.1 Introduction ......................................................... 170
6.2 Objectives .......................................................... 171
6.3 Study Design ........................................................ 172
  6.3.1 General ......................................................... 172
  6.3.2 Design considerations ........................................ 173
6.4 Subjects, Protocol and Methods ................................. 175
  6.4.1 Subjects ........................................................ 175
  6.4.2 Protocol ......................................................... 176
  6.4.3 Drug Administration and Dosages ......................... 176
  6.4.4 Schedule ....................................................... 179
  6.4.5 Clinical measurements and procedures .................... 180
  6.4.6 Laboratory methods ......................................... 181
  6.4.7 Data analysis ................................................ 182
6.5 Results .............................................................. 182
CHAPTER 7

DISCUSSION ................................................................. 222

7.1 The exploratory clinical development of teicoplanin using a
surrogate marker ...................................................... 223

7.2 Future clinical development of teicoplanin as a treatment for sickle
cell disease ............................................................... 224

7.3 Teicoplanin as an immunomodulator ............................... 226

7.3.1 Immunological effects of teicoplanin in vitro ................... 228

7.3.2 Immunological effects of teicoplanin in vivo .................... 228

7.3.3 Antiviral effects of teicoplanin .................................. 228
7.3.4 Antitumour effects of tacarrelol
7.3.5 Potential clinical uses for tacarrelol as an
immunosuppressant
7.4 Conclusions

S U B L I B R A D Y

APPENDICES - Papers arising from original work presented in this thesis.

Appendix A.
The pharmacokinetics, tolerability and pharmacodynamics of tacarrelol
(S9C80, 4(2-formyl-3-hydroxyphenoxymethyl)benzoic acid), a potential
antisickling agent, following oral administration to healthy volunteers.

Appendix B.
Pharmacokinetics and pharmacodynamics of multiple oral doses of tacarrelol,
an antisickling agent, in healthy volunteers.
ABSTRACT

1. Sickle cell disease is a family of inherited haemoglobinopathies resulting from a point mutation in the gene coding for the β-chain of haemoglobin, resulting in the substitution of valine for glutamate at the sixth amino acid residue on the β-chain. Sickle haemoglobin (HbS) containing the abnormal β-chain functions much like normal haemoglobin (HbA) when oxygenated, but when de-oxygenated, HbS polymersises into helical fibres which distort the normal discoid shape of the red blood cell into a "sickle" shape. It is widely believed that a treatment which prevents polymerisation of deoxy HbS in vivo would improve the clinical manifestations of the disease.

2. Tucuareol (4(2-formyl)-3-hydroxy-phenoxymethyl/phenolic acid) was designed to bind preferentially to the oxy-conformation of human haemoglobin at a site between the amino terminal residues of the α-subunits, stabilising haemoglobin in the oxy-conformation. This results in a left-shift of the haemoglobin oxygen saturation curve (OSC), increasing the proportion of oxy-Hb at any given low oxygen tension, thereby offering the possibility of preventing sickling in vivo.

3. A new surrogate marker (%MOD) had previously been developed to assess the effect of tucuareol and related compounds in man. %MOD is defined as the proportion of haemoglobin molecules reacted with haemoglobin in a high affinity form. It is measured by comparing observed OSC's ex vivo with a series of template curves ranging from 0%MOD to 100%MOD in 5% increments. From analysis of the kinetics of formation of the sickle polymer it was estimated that 15 - 30 % MOD would be required for the effective prophylaxis of the manifestations of the disease.
4. This thesis describes the exploratory clinical development of tucaresol, consisting of the three studies performed in man to the date of writing. The first human study with tucaresol was of open, single-dose, ascending-dose, crossover design in 9 healthy male volunteers. Dosages ranged from 200 - 3000 mg. Peak concentrations in plasma and erythrocytes were linearly related to dose but were approximately an order of magnitude higher in erythrocytes than in plasma. There was evidence of distribution of drug from plasma to erythrocytes over 24 hours from dosing. Terminal elimination half-life was approximately twice as long from plasma than from erythrocytes, with mean values after the top dose of 229 and 151 h respectively. At the highest dose, peak %MOD was between 19-26%. The drug was well tolerated, with only minor gastrointestinal discomfort at high doses. There were no clear effects on routine haematology and biochemistry, platelet aggregation, resting or exercise heart rates or blood pressure.

5. The second human study was of placebo-controlled, parallel-groups design in 12 healthy male volunteers. The 8 subjects on active drug received three doses of tucaresol at 48 hour intervals. The first was estimated by body weight to achieve 15 % MOD, and the subsequent two doses were individually titrated to produce 22 and 32.5 % MOD. Mean peak achieved %MOD was 34%. Pharmacokinetics were similar to those in the previous study. There was a small increase in heart rate after exercise in the tucaresol group compared to the placebo group. A major unexpected finding was the development of a syndrome of rash, fever and tender cervical lymphadenopathy with onset 7-10 days from dosing suggesting an immune mechanism.
6. The third human study was of double-blind, placebo-controlled, parallel-groups design in 12 stable patients with sickle cell disease. Cumulative doses were progressively reduced from 6400 and 4000 mg over 10 days in the first pair of subjects, to 3000 in the next four patients and 500 mg in the last pair because of rapid rise in haemoglobin and adverse experiences at the higher doses. The pharmacokinetics of sucresol were similar to those in healthy volunteers, but there was a trend for reduced clearance in women compared to men. Peak % MOD values were 21 and 24%. Three subjects developed fever and tender cervical lymphadenopathy within 7-10 days from the start of dosing. Two were treated with prednisolone with prompt resolution of symptoms. In all six subjects attaining >10% MOD there was evidence of an antisticking effect of sucresol, evidenced by rises in haemoglobin, falls in irreversibly sickled cell counts, plasma lactate dehydrogenase and bilirubin.

7. Subsequent in vitro and animal studies investigated the possible effects of sucresol on the immune system. Sucresol was found to have powerful immunostimulant properties with antiviral and antimicrobial effects. The likely mechanism was the formation of Schiff's-base adducts with helper T cells mimicking the Schiff's-base mediated communication between antigen-presenting cells and helper T cells. Further evaluation of sucresol in chronic viral infections and possibly cancer is warranted.

8. This thesis demonstrates that rational drug design may be an efficient way of selecting potential therapeutic candidates. A mechanistically-based surrogate may be very helpful in comparing pharmacology and kinetic studies between animals and man.
and help design dosage regimens. However, the clinical pharmacist in exploratory development needs to look for effects other than those expected.