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

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ABSTRACT

1. Sickle cell disease is a family of inherited haemoglobinopathies resulting from a point mutation in the gene coding for the ß-chain of haemoglobin, resulting in the substitution of valine for glutamate as the sixth amino acid residue on the ß-chain. Sickle haemoglobin (HbS) containing the abnormal ß-chain, functions much like normal haemoglobin (HbA) when oxygenated, but when de-oxygenated, HbS polymerises 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. Tucaresol (4(2-formyl-3-hydroxy-phenoxymethyl)benzoic 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 tucaresol and related compounds in man. %MOD is defined as the proportion of haemoglobin molecules reacted with haemoglobin to 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. Doses ranged from 200 - 3600 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 289 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 pressures.

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 25 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 tucaresol were similar to those in healthy volunteers, but there was a trend for reduced clearance in women compared to men. Peak % MOD values were 23 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 antisickling effect of tucaresol, 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 tucaresol on the immune system. Tucaresol was found to have powerful immunostimulant properties with antiviral and antitumour 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 tucaresol 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 pharmacologist in exploratory development needs to look for effects other than those expected.