Pharmacokinetics and pharmacodynamics of tucaresol, an antisickling agent, in healthy volunteers

P. E. ROLAN*, A. J. MERCER, R. WOOTTON & J. POSNER

Department of Clinical Pharmacology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, UK

- 1 Tucaresol is an orally administered antisickling agent which increases the oxygen affinity of haemoglobin.
- 2 The pharmacokinetics, effects on moderate graded exercise and psychometric performance of tucaresol were examined in a double-blind, placebo-controlled, parallel groups study in 12 healthy men.
- 3 Three doses of tucaresol were given at 48 h intervals intended to modify 15, 25 and 32.5% of a subject's haemoglobin to a high affinity form (%MOD).
- 4 Mean peak %MOD was 34%. Mean $C_{\max}$ values in plasma and erythrocytes were 81.4 and 1459 $\mu\text{g ml}^{-1}$, respectively.
- 5 Heart rate, compared with baseline, increased in the tucaresol group with the greatest changes at the highest %MOD and workload. There were no differences between groups in psychometric test performance.
- 6 Three volunteers on active drug developed fever, rash and tender cervical lymphadenopathy with onset 7–10 days from the start of dosing, suggesting an immune mechanism.
- 7 The acute increase in oxygen affinity with tucaresol is physiologically well-tolerated, but the utility of tucaresol in the management of sickle cell disease will depend on the identification of a dosing regimen with a lower incidence of drug allergy.

Keywords tucaresol anti-sickling agent sickle cell disease pharmacokinetics pharmacodynamics

Introduction

Sickle cell disease is due to a family of inherited haemoglobinopathies characterised by an abnormal haemoglobin, haemoglobin S, which polymerises when deoxygenated [1]. Polymerisation of haemoglobin S results in rigid distorted red cells, with the characteristic 'sickle' shape and leads to the micro-vascular obstruction which is thought to be the pathological basis for the clinical course of the disease [1]. At present there is no licensed specific therapy in clinical practice, and treatment is primarily supportive [2]. As the rate of sickling is dependent on the concentration of insoluble deoxy-haemoglobin S, decreasing the deoxy-haemoglobin S concentration relative to the oxy- conformation would be expected to confer therapeutic benefit. It has been postulated that this could be achieved by increasing the oxygen

affinity of haemoglobin, i.e. 'left-shifting' the haemoglobin-oxygen saturation curve (OSC) [3].

Tucaresol, (589C80; 4[2-formyl-3-hydroxyphenoxy-methyl] benzoic acid), is a substituted benzaldehyde which interacts stoichiometrically with haemoglobin to produce a haemoglobin tetramer with increased oxygen affinity [4, 5]. The oxygen affinity of a whole blood sample is progressively increased by tucaresol in a concentration-dependent manner. This is due to an increasing proportion of molecules reacted to the high-affinity form, a term previously defined as the 'percent modification' denoted '%MOD' [4]. Evidence for an anti-sickling action of tucaresol *in vitro* comes from increased filterability of suspensions of sickle erythrocytes at low oxygen concentrations in a concentration-dependent manner [6]. It is estimated,

by analysis of the kinetics of sickle haemoglobin polymer formation, that for effective therapy it will be necessary to modify between 20 and 30% of a patient's haemoglobin to the tucaresol-reacted, high oxygen affinity form (20–30% MOD) [7–9].

We have previously reported the pharmacokinetics, pharmacodynamics and tolerability of escalating single oral doses of tucaresol in healthy volunteers [10]. Doses were from 200 to 3600 mg and peak %MOD at the highest dose ranged from 19–26%. The drug was well tolerated but with minor gastrointestinal discomfort at the highest dose. Tucaresol was well absorbed, and was concentrated in erythrocytes. The value of $t_{\max}$ in erythrocytes (median 24 h) was later than that in plasma (median 6 h). Elimination half-life from erythrocytes (mean 151 h at 3600 mg) was shorter than that from plasma (mean 289 h). In this first study the full anticipated therapeutic range (15–30 %MOD) was not spanned. We now report results from a larger study in which higher %MOD levels were achieved.

Methods

Study design

The study was of placebo-controlled double-blind parallel groups design, eight volunteers receiving tucaresol and four placebo. The twelve volunteers were studied as four cohorts of three, each cohort having two volunteers on active drug and one on placebo. Volunteers resided in an Investigation Unit from study days 0–6. Three doses of tucaresol or matching placebo were administered after an overnight fast at approximately 08.00 h on days 1, 3 and 5. It was intended that peak %MOD after each dose would be 15, 25 and 32.5% respectively. The initial dose was based on body weight and the subsequent two doses by individual titration. On days 0 (baseline), 2, 4 and 6 the cardiovascular response to moderate graded exercise and psychometric performance were assessed. Blood samples for assay of tucaresol in plasma and lysed whole blood were taken from all volunteers on days 0–7 inclusive and days 9, 11, 13, 17, 20, 27, 34, and 41. The random code was then broken and further samples were taken only from those who received tucaresol on days 48, 55, 62, 69 and 76. Samples were taken for full blood count and biochemistry at the same times omitting days 2, 4 and 6. Samples for blood coagulability (prothrombin time and activated partial thromboplastin time) were taken on days 0, 6 and 41.

Subjects

Volunteers were healthy male Caucasians of mean age 27 (range 20–34) years and mean weight 82 (range 73–98) kg. Volunteers underwent full physical examination and ECG at screening, and provided written consent. All subjects were of haemoglobin A phenotype.

Drug and dosing

Tablets of 200 and 400 mg of tucaresol and matching placebo were supplied by The Wellcome Foundation, Dartford, Kent, UK. The initial dose for each subject was 35 mg kg⁻¹ (rounded to the nearest 200 mg unit dose) and subsequent doses were calculated from the previous day's %MOD value for each subject. The dose was given after an overnight fast. Each dose was calculated and dispensed by a pharmacist and checked by the scientist measuring the %MOD levels. These staff were aware of the random code but were not otherwise involved in the study and kept separate records from the clinical investigators to maintain the double-blind. Similar adjustments to numbers of placebo tablets to those necessary for active treatment were also made in order to protect the blinding.

Exercise and cardiovascular parameters

The cardiovascular response to moderate graded exercise was investigated in two body positions. Exercise was performed either supine (at 7.5° to the horizontal) or sitting, at rest (0 Watts (W)) and at incrementing work rates of 50, 85 and 120 W for 3 min at each level. Exercise was suspended for 1 min between ascending work rates to allow blood pressure, heart rate (Hewlett Packard HP78325A) and respiration to be monitored. Cardiac output and stroke volume were measured for 10 s immediately at the end of each work level by bioimpedance plethysmography (BOMED NCCOM3, Bomed Medical Manufacturing, Irvine, California). Within the last 30 s of each work level the perceived exertion was recorded using the Borg scale [11].

Psychometric tests

Choice reaction time [12], the Stroop test [13], a mood adjective checklist and a visual analogue mood rating scale [14] were performed using a personal computer with a touch-sensitive screen. Subjects underwent several training sessions until they had apparently been trained to plateau.

Laboratory methods

The assay of tucaresol in plasma and lysed whole blood and determinations of %MOD coagulation were performed as reported previously [10]. Full blood counts were measured with a Sysmex E500 analyser (Toa Medical, Japan) with a between-batch precision of <5%, and plasma biochemistry was analysed on a Hitachi 717 system with between-batch precision of <3%. Blood counts and plasma biochemistry were analysed in the Department of Clinical Pathology at Wellcome Research Laboratories using daily quality controls.

Pharmacokinetics

Non-compartmental pharmacokinetic analysis was performed using methods described previously [10].

The elimination rate constant k was calculated from the major portion of the elimination part of the log drug concentration-time curve. For calculation of oral clearance, the total dose was divided by AUC. Erythrocyte tucaresol concentrations were calculated from the plasma and whole blood drug concentrations and the haematocrit [10].

Statistical analysis

Differences between treatments in change from baseline (day 0) for each pharmacodynamic variable were assessed using analysis of variance. Ninety-five percent confidence intervals were calculated for the differences between treatments (tucaresol/placebo) in change from baseline at each time point.

Results

Haemoglobin modification

Peak %MOD occurred in most cases on Day 6. The mean peak value was 34% and ranged from 32–38%. Achieved and target %MOD values were close; a comparison is presented in Figure 1. Modification was not detectable in any subject after day 35.

Tolerability and adverse experiences

Diarrhoea was reported by three subjects from the first two cohorts on the first day of dosing. To avoid such gastrointestinal symptoms, subsequent doses were split into two fractions of approximately equal size. The first fraction was administered at approximately midnight, 8 h before the second fraction which was given at the scheduled time. There was one further report of diarrhoea on day 1; this subject also vomited. Three volunteers from separate cohorts on active drug developed a similar syndrome of adverse experiences characterised by fever, rash and tender cervical lymphadenopathy. The onset of these symptoms was between days 7 to 10. The highest temperature recorded was 39.1°C. The rash was of red blanching macules on the trunk and limbs but not in the oral cavity and was not itchy. The cervical lymph nodes were moderately enlarged and their tenderness was marked; one subject was disinclined to turn his head from side to side. The symptoms substantially settled spontaneously within 3–4 days but the volunteers felt tired for approximately 2 weeks. Extensive paired (screen, follow-up) viral serology (influenza A & B, adenovirus, mycoplasma, herpes simplex, psittacosis, measles, mumps, cytomegalovirus, Q fever, Epstein-Barr capsid and nuclear antigen antibodies, hepatitis A) showed no evidence of recent infection.

Haematology and biochemistry

There were no differences in haematological or coagulation variables between groups. Mildly elevated

Figure 1 Target (○) and achieved (●) values of percent haemoglobin modification (%MOD) and cumulative dose of tucaresol (□).

transaminases (in general to within double the upper limit of the normal range) were reported in five subjects on active drug and two on placebo. The greatest increase was a value of alanine aminotransferase of 219 u l^{-1} on day 7 (normal range $<45 \text{ u l}^{-1}$). As this was thought to be potentially clinically significant, the code for this subject only was broken and he was found to be receiving placebo. He was questioned about recent illnesses, drug or chemical exposure in the workplace and no explanation was found.

Cardiovascular data

At rest (0 W), there were no significant changes from baseline in either treatment group. On exercise, heart rates in the placebo group remained comparable with those at the same exercise level on day 0 but there was a time-dependent (and hence %MOD-dependent) increase in heart rate on exercise compared with the same work rate at baseline in the tucaresol group and at the highest work rates. Following supine exercise, there were significant differences on day 2 between treatments (tucaresol/placebo) at 120 W (95% CI 9–22 beats min^{-1}); on day 4, significant differences were at 50 and 120 W (95% CI 3–20 and 10–27 beats min^{-1} respectively) and on day 6, significant differences were at 50, 85 and 120 W (95% CI 0–33, 8–24 and 13–36 beats min^{-1} respectively). Following seated exercise there were similar trends for higher heart rates in the tucaresol group especially at higher workloads. The mean heart rates are shown in Figures 2a (supine) and 2b (sitting). There were no consistent trends for differences between treatments in systolic or diastolic blood pressure, respiration rate, cardiac stroke or output volume. There were no clinically significant changes on resting ECG.

Psychometric tests

There were no trends for differences between treatments in any of the psychometric tests.

Pharmacokinetics

The mean log concentration-time curves of tucaresol in plasma and erythrocytes are shown in Figure 3,

Figure 2 Mean change from baseline (Day 0) in heart rate following a) supine exercise and b) sitting exercise. * = $P < 0.05$. ● placebo, ■ tucaresol.

Figure 3 Mean plasma (●) and erythrocyte (■) tucaresol concentrations and the %MOD-time curve (▲).

and a summary of pharmacokinetic parameters in Table 1. The maximum concentrations of tucaresol in plasma and erythrocytes occurred on day 5 or 6 with mean (s.d.) values of 81.4 ± 4.1 and $1459 \pm 162 \mu\text{g ml}^{-1}$, respectively. Inspection of the individual log concentration-time curves showed the elimination phase to be triphasic, with the second phase being faster than either the first or third. Half-life was

determined from this second portion as it represented the majority of the curve. Mean (s.d.) half-life was 257 ± 39 h from plasma and 157 ± 29 h from erythrocytes. The ratio of tucaresol concentration in erythrocytes/plasma declined progressively from a peak of 18.9 on day 6 to 1.0 on day 56. Figure 4 shows a plot of the mean value of this ratio against plasma drug concentration.

Discussion

The major unexpected finding of this study was the occurrence of adverse experiences of fever, rash and tender cervical lymphadenopathy in three of the eight volunteers who received tucaresol. These effects were not predicted by animal studies or observed in the nine volunteers in the previous study [10]. Initially the cause was thought to be viral, but the consistent onset from start of dosing, the fact that the three affected volunteers came from separate cohorts in the Investigation Unit at separate times and the absence of similar symptoms in the placebo group led to the conclusion that these symptoms were drug-related. The mechanism of the adverse experiences is most un-

Table 1 Pharmacokinetic and %MOD data

Subject	Dose (mg)	C_{max} ($\mu\text{g ml}^{-1}$)	t_{max} (h)	Plasma		CL_o (ml min^{-1})	C_{max} ($\mu\text{g ml}^{-1}$)	Erythrocyte		$t_{1/2}$ (h)	%MOD		
				AUC ($\mu\text{g ml}^{-1} \text{h}$)	$t_{1/2}$ (h)			AUC ($\mu\text{g ml}^{-1} \text{h}$)	t_{max} (h)		Max. %MOD	t_{max} (h)	$t_{1/2}$ (h)
2	5556	85.7	120	42844	307	2.16	1540	120	593178	214	34	120	199
3	6555	83.1	120	37849	247	2.89	1670	120	501109	149	34	120	187
4	6147	83.2	96	29982	199	3.42	1195	120	353930	136	33	120	168
5	6158	77.3	96	34037	294	3.02	1458	96	484546	151	34	120	216
8	5158	76.4	96	34679	271	2.48	1371	144	476729	127	32	120	209
9	6953	81.9	120	41043	284	2.82	1638	96	582369	178	38	120	193
10	5760	76.9	120	35988	212	2.67	1308	120	410120	165	33	120	162
12	5964	86.9	120	36835	241	2.70	1490	120	486425	133	35	144	187
Mean	6031	81.4	108*	36657	257	2.77	1459	132*	486051	157	34.1	120*	190
s.d.	563	4.1	—	4049	39	0.37	162	—	79544	29	1.8	—	19

C_{max} : maximum concentration. t_{max} (*median): time of maximum concentration. AUC: area under the concentration-time curve to infinity. $t_{1/2}$: dominant half-life. CL_o : oral plasma clearance.

Figure 4 Erythrocyte/plasma concentration ratio of tucaresol as a function of plasma concentration of tucaresol.

likely to be due to the increase in oxygen affinity of haemoglobin. Healthy volunteers and cancer patients who received valeresol (BW12C; (5[2-formyl-3-hydroxyphenoxy]pentanoic acid), an analogue of tucaresol with a similar effect on oxygen affinity, but a much shorter plasma half-life, have not reported similar symptoms despite peak %MOD levels in excess of 40% [15, 16]. Also, individuals with naturally occurring high-affinity haemoglobins are usually asymptomatic. Rather, the nature of the symptoms, the timing 7–10 days from first exposure to the drug and the frequent presence of activated/atypical lymphocytes in the peripheral blood film suggest drug allergy. It is possible that a gradual introduction to the drug, rather than a rapid loading as used in this study, might decrease the risk of adverse experiences as has been found with allergy to anticonvulsants [17–19].

The cardiovascular response to exercise was included in this study to assess whether the increased oxygen affinity of tucaresol-reacted blood would be well-tolerated on an ambulatory basis since it might result in a decreased oxygen delivery per unit volume of blood. A likely compensatory response to maintain tissue oxygen delivery would be increased blood flow

through increased cardiac output. However, no treatment related differences in cardiac output were detected but values of cardiac output and stroke volume from the NCCOMM 3 were very variable. It has been reported that although the bioimpedance method has low variability when the subject is at rest, variability increases with exercise possibly due to the software treating electrical activity from chest muscles as cardiac events [20]. However, evidence in support (although it cannot be conclusive) of an increase in cardiac output comes from the heart rate data. Although there were no treatment-related differences in heart rate at rest, subjects receiving tucaresol had increases in heart rate from the baseline period after exercise. This result is similar to that from a study with valeresol in which resting heart rate was significantly increased only at the highest dose producing 40% MOD, although lower doses and %MODs produced higher heart rates than placebo after exercise [15]. Thus the effect of increased oxygen affinity on heart rate may only be apparent under conditions of increased oxygen demand. Despite the treatment-related increase in exercise heart rate the exercise tests were well-tolerated and there was no increase in perceived exertion. Thus, this degree of increase in oxygen affinity is likely to be well tolerated at rest and on exercise. This is supported by a placebo-controlled double-blind study with valeresol in which volunteers exercised at a work rate of approximately 180 beats min^{-1} at 0, 15.7 and 31.5 %MOD. Volunteers were able to complete these near-maximal exercise tests satisfactorily, and there was little change in oxygen consumption at an arterialised plasma lactate of 3 mM [21].

Psychometric tests were included because of the theoretical risk of reduced oxygen delivery per unit volume of blood having effects on cortical function. Examples of motor and cognitive function were used. Choice reaction time was selected as it had previously been shown to be affected by hypoxia [22, 23]. Although subjects had been trained to apparent plateau, some improvement in test performance

occurred in both treatment groups between days 0 and 2 suggesting that the training had not been adequate. Although the groups sizes were small, no treatment-related effects were observed and this is consistent with the absence of reports on adverse experiences referable to the CNS.

As has been mentioned previously [10], the pharmacokinetics of tucaresol are complex. We have commented on the unusual feature of the apparent terminal elimination half-life in a tissue (erythrocyte) being shorter than from plasma. This would suggest that steady state had not been reached and so in this study sampling was continued for longer. Figure 4 is a plot of the erythrocyte to plasma tucaresol concentration ratio against plasma tucaresol concentration, showing an approximately linear relationship which appears to fall off at higher levels. A similar depen-

dency of this ratio on plasma concentration was seen in the previous dose escalation study in terms of $C_{\max}$ values and probably arises from complex binding patterns to both haemoglobin and plasma proteins with different numbers and affinities of binding as observed for valeresol [24]. The relative persistence of tucaresol in plasma may be due to very tight binding of tucaresol to a plasma protein. This drug would not be available for elimination but must be available to be assayed as tucaresol and, therefore, the binding must be reversible by the acidification used in the extraction procedure in the assay. The presence of a protein-drug complex may be relevant to the development of drug allergy. Whether tucaresol is a suitable drug for the management of sickle cell disease may depend on a dosing strategy which would minimise the incidence of drug allergy.

References

- 1 Dean J, Schechter AN. Sickle cell anaemia. Molecular and cellular bases of therapeutic approaches. Part 1. *New Engl J Med* 1978; **299**: 752-763.
- 2 Platt AS. Is there treatment for sickle cell anaemia? *New Engl J Med* 1988; **319**: 1479-1480.
- 3 Dean J, Schechter AN. Sickle cell anaemia. Molecular and cellular bases of therapeutic approaches, Part 3. *New Engl J Med* 1978; **299**: 863-870.
- 4 Beddell CR, Goodford PJ, Kneen G, White RD, Wilkinson S, Wootton R. Substituted benzaldehydes designed to increase the oxygen affinity of human haemoglobin and inhibit the sickling of sickle erythrocytes. *Br J Pharmacol* 1984; **82**: 397-407.
- 5 White RD, Wootton R. 589C80, a potent, long-acting left-shifter of the oxygen saturation curve of haemoglobin *in vivo* in the rat. *Br J Pharmacol* 1989; **98**: 772.
- 6 Keidan AJ, Sowter MC, Johnson CS, Marwah SS, Stuart J. Pharmacological modification of oxygen affinity improves deformability of deoxygenated sickle erythrocytes: a possible therapeutic approach to sickle cell disease. *Clin Sci* 1989; **76**: 357-362.
- 7 Sunshine HR, Hofrichter J, Eaton WA. Requirements for therapeutic inhibition of sickle haemoglobin gelation. *Nature* 1978; **275**: 238-240.
- 8 Franklin IM, Rosemeyer MA, Huehns ER. Sickle cell disease: the proportion of liganded haemoglobin needed to prevent crises. *Br J Haematol* 1983; **54**: 579-587.
- 9 Keidan AJ, White RD, Huehns ER, Franklin IM, Joy M, Stuart J. Effects of BW12C on oxygen affinity of haemoglobin in sickle cell disease. *Lancet* 1986; **i**: 831-834.
- 10 Rolan PE, Parker JE, Gray SJ, *et al.* The pharmacokinetics, tolerability and pharmacodynamics of tucaresol (589C80; 4[2-formyl-3-hydroxyphenoxymethyl] benzoic acid), a potential anti-sickling agent, following oral administration to healthy subjects. *Br J Clin Pharmacol* 1993; **35**: 419-425.
- 11 Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehab Med* 1970; **2-3**: 92-98.
- 12 Hindmarch I. *Psychomotor function and psychoactive drugs*. Central Nervous System eds Lader M, Richens A. 1981 (Macmillan).
- 13 Stroop JR. Studies of interference in serial verbal reactions. *J exp Psychol* 1935; **18**: 643-662.
- 14 Bond A, Lader M. The use of visual analogue scales in rating subjective feelings. *Br J med Psychol* 1974; **47**: 211-218.
- 15 Nicholls AJ, Parker JE, Posner J. Increased Hb-O₂ affinity induced by BW12C in healthy volunteers: effect on heart rate. *Br J Clin Pharmacol* 1989; **28**: 215P.
- 16 Ramsey JRS, Bleehen NM, Dennis I, *et al.* Phase I study of BW12C in combination with mitomycin C in patients with advanced gastrointestinal cancer. *Int J Radiation Oncology Biol Phys* 1992; **22**: 721-725.
- 17 Wilson JT, Hojer B, Rane A. Loading and conventional dose therapy with phenytoin in children: Kinetic profile of parent drug and main metabolite in plasma. *Clin Pharmacol Ther* 1976; **20**: 48-58.
- 18 Wilson JT, Hojer B, Tomson G, Rane A, Sjoqvist F. High incidence of a concentration-dependent skin reaction in children treated with phenytoin. *Br med J* 1978; **1**: 1583-1586.
- 19 Chadwick D, Shaw MDM, Foy P, Rawlins MD, Turnbull DM. Serum anticonvulsant concentrations and the risk of drug induced skin eruptions. *J Neurol Neurosurg Psychiatr* 1984; **47**: 642-644.
- 20 Smith SA, Russell AE, West MJ, Chalmers J. Automated non-invasive measurement of cardiac output: comparison of electrical bioimpedance and carbon dioxide rebreathing techniques. *Br Heart J* 1988; **59**: 292-298.
- 21 Nicholls A, Parker JE, Posner J. Effect of BW12C on lactate levels during exercise in healthy volunteers. *Br J Clin Pharmacol* 1989; **28**: 747P.
- 22 Schaffler and Klausnitzer W. Randomised, placebo-controlled, double-blind crossover study on the anti-hypoxic effects of piracetam using psychophysiological measures in healthy volunteers. *Drug Res* 1988; **38**: 288-291.
- 23 Schaffler K, Hormann E, Arnold H. A pilot study to evaluate the effect of acute and long-term administration of cyclandelate on the vigilance of volunteers submitted to hypoxic conditions. *Drug Res* 1981; **31**: 1032-1035.
- 24 Merrett M, Stammers DK, White RD, Wootton R, Kneen G. Characterisation of the binding of the anti-sickling compound 'BW12C' to haemoglobin. *Biochem J* 1986; **239**: 387-392.

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