A PHARMACOKINETIC AND TOLERANCE STUDY OF Ro13-9904, A NEW CEPHALOSPORIN ANTIBIOTIC

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- 1 Six healthy male volunteers received a total of 2500 mg of a new cephalosporin antibiotic Ro13-9904 by intramuscular injection in five divided doses at intervals of 12 h.
- 2 No significant systemic side-effects were observed and this was confirmed haematologically and biochemically.
- 3 The drug was distributed following intramuscular injection reaching a mean peak plasma concentration of 55 $\mu\text{g ml}^{-1}$ (range 46–66) 1 to 2 h after the first injection.
- 4 Monoexponential elimination of drug was demonstrated. No significant difference was recorded in the plasma half-life after the initial dose (mean 6.7 h) and at steady state (mean 6.7 h). The half-life is long compared with other cephalosporin antibiotics.
- 5 On the basis of the observed half-life, steady state should be reached within 48 h. A mean peak plasma concentration of 74 $\mu\text{g ml}^{-1}$ (range 65–87) was recorded at steady state. Steady state plasma concentrations of Ro13-9904 with a dose of 500 mg every 12 h may be predicted from the pharmacokinetics of a single dose.

Introduction

Ro13-9904 (Rocephin[®]) is the disodium salt of (6R, 7R)-7-[2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-[[[(2, 5-dihydro-6-hydroxy-2-methyl-5-oxo-as-triazin-3-yl) thio] methyl]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid. It is one of the new generation of cephalosporins possessing both enhanced stability to β -lactamase and a broad spectrum of antibacterial activity (Shannon *et al.*, 1980; Wise *et al.*, 1980). The extremely good activity against most enterobacteriaceae, including gentamicin resistant strains, *Haemophilus influenzae*, *Neisseriae gonorrhoea* and *N. meningitidis* are of particular interest.

Ro13-9904 is only poorly absorbed following oral administration, but is well tolerated following single intramuscular or intravenous administration. The compound differs from currently available cephalosporins in its uniquely long half-life of 6.5–7 h (Seddon *et al.*, 1980). On the basis of these early studies a therapeutic dosage regime of twice daily administration is proposed.

Toxicology studies have shown no evidence of renal toxicity nor local intolerance, and early studies in patients have confirmed the activity and safety of this compound.

The purpose of this study was to determine the plasma concentrations of Ro13-9904 following in-

tramuscular administration of repeated doses twice daily to healthy volunteer subjects, and also to study in detail the safety of such a dose regime.

Methods

Six male volunteers (mean age 25.7 years, range 19–43), one of whom was a maturity onset diabetic controlled by diet alone, were selected on the basis of full normal medical examination, including ECG, haematology and clinical chemistry screen. Each agreed to participate after a full explanation of the study had been given. None had received medication in the 2 weeks prior to the study. No subject had hypersensitivity to β -lactam antibiotics and none had a history of bowel disorder or abnormal liver or renal function. Volunteers were permitted a normal diet with fixed standard meal times during the study. Strenuous exercise was prohibited and smoking limited to a maximum of 10 cigarettes a day. Fluid intake was at least 1500 ml daily.

The subjects were admitted to hospital for 6 days. Injections were given at 08.30 h and 20.30 h on each of the first 4 days. After two saline placebo injections (3.0 ml) on day 1, Ro13-9904 was given by deep intramuscular injection (500 mg made up to 3 ml with 1% lignocaine in saline) twice daily on days 2 and 3

and once on day 4. The second and final injection on day 4 was saline placebo (3.0 ml). Care was taken to ensure that the injection site was different on each occasion and that the buttocks were used alternately.

Physiological tests

Body weight was recorded each morning at the same time and heart rate, blood pressure (lying and standing), pupil reaction and temperature at regular intervals over the 5 days. A full medical examination and ECG was performed on completion of the study.

Haematological screen

Full blood count was performed at 0, 2 and 6 h after the first injection on days 1, 2 and 4, and at 0 h on days 3, 5 and 6. Electrolytes, urea and liver function tests were performed at the same times. A Coombs' test was performed at the start and end of the study.

Urine analysis

All urine passed during days 1-5 was collected. Urine analysis (Labstix), a clinical chemistry screen and *N*-acetyl- β -glucosaminidase (NAG) activity (Tucker *et al.*, 1975) - a measure of the extent of renal damage - were measured on urine aliquots from 0-2, 2-4, 4-6, 6-8, 8-12 and 12-24 h on days 1-4 inclusive. The first 12 h urine from day 5 was pooled and the same tests performed on an aliquot.

Plasma concentrations

Heparinized blood (10 ml) was collected through an indwelling venous cannula 0, 15, 30, 45, 60 and 90 min, 2, 3, 4, 6, 8, 10 and 12 h after the first injection on days 2 and 4. Samples were also taken through a syringe at 0 and 1 h after the first injection on day 3. Plasma was stored at -20°C to await analysis by a modification of the high performance liquid chromatographic method of Trautman & Haefelfinger (1980) using the reverse phase system of Lichrosorb RP18 column (150×3.2 mm i.d. steel), $5\mu\text{M}$ particle size, and mobile phase consisting of 4 g hexadecyltrimethylammonium bromide, 500 ml acetonitrile and 50 ml phosphate buffer (pH 7.0) made up to litre with distilled water, flow rate 1.5 ml min^{-1} . 5-Sulphosalicylic acid was used as internal marker.

Urine concentrations

Having recorded the total volume, aliquots from urine passed from 0-2, 2-4, 4-6, 6-8, 8-12 and 12-24 h on days 2 and 4 and from 0-12 h on days 3

and 5 were stored at -20°C to await analysis. The method was as described above but with a mobile phase consisting of 3.8 g tetrapentylammonium bromide, 200 ml acetonitrile and 38 ml phosphate buffer (pH 7.8) made up to 1 l with distilled water, and benzoic acid as internal marker.

Pharmacokinetic analysis

Having determined that drug elimination was monoexponential, plasma half-lives ($T_{1/2}$) were obtained by linear least squares regression analysis of all the data points following the peak concentration.

Following the initial dose of drug (i.e. day 2 of the study) the area under the plasma concentration versus time plot from zero to infinite time ($\text{AUC}_{0-\infty}$) was calculated using the expression $\text{AUC}_{0-\infty} = \text{AUC}_{0-12\text{h}} + \text{AUC}_{12-\infty}$. $\text{AUC}_{0-12\text{h}}$ was measured using the "cut and weigh" method (Gibaldi & Perrier, 1975) and $\text{AUC}_{12-\infty}$ was calculated using the equation

$$\text{AUC}_{12-\infty} = \frac{C_{12} \times T_{1/2}}{0.693}$$

where c_{12} is the plasma concentration 12 h after dosing.

The cut and weigh method was used to calculate the area under the plasma level curve following the last dose of drug (day 4)

$$\text{AUC}_{(4)} = \int_{0\text{h}}^{12\text{h}} C_{\infty}(t) \cdot dt.$$

The apparent volume of distribution after the initial dose was calculated using

$$V_{\text{d.i.d.}} = \frac{F \cdot \text{Dose} \cdot T_{1/2\text{i.d.}}}{0.693 \cdot \text{AUC}_{0-\infty}}$$

assuming complete drug availability (i.e. $F = 1$).

Total body clearance was calculated using the expression

$$Q_B = \frac{F \cdot \text{Dose}}{\text{AUC}_{0-\infty}}$$

Results

Apart from the one subject who was a maturity onset diabetic controlled by diet alone, medical examination on all volunteers was otherwise normal. All ECGs were normal on completion of the study.

In four subjects local pain at the injection site, not seen with saline, was measured only in minutes. Two subjects experienced discomfort of longer duration particularly after the fourth and fifth drug injections. In one of these subjects similar discomfort was seen after saline injection.

No serious systemic side-effect was noticed though three subjects complained of looseness rather than frequency of bowel action on the fourth and fifth days of the study. In each case this resolved spontaneously within a further 2 days. No significant changes were observed in temperature, pulse, blood pressure, pupil reaction or weight throughout the study.

There was no change in any haematological parameter throughout the study and Coombs' test was negative in all subjects on both occasions. Electrolytes, urea, creatinine and liver function tests were normal throughout the study in all subjects. In most subjects however a modest rise in creatine phosphokinase was seen after the intramuscular injections of cephalosporin. This did not occur with the saline placebo injections on day 1. Urine analysis throughout the study was entirely normal in four subjects. One subject had traces of proteinuria on three occasions though testing was otherwise completely normal. Another subject, the diabetic, showed intermittent glucose of varying amounts but no ketones. His urine was otherwise normal.

No abnormality in urinary electrolytes, urea or creatinine was seen in any subject during the course of the study. Urinary NAG activity showed no individual values in excess of $300 \text{ nmol h}^{-1} \text{ mg}^{-1}$ creatinine which would indicate renal damage.

Pharmacokinetic data for the individual subjects are shown in Table 1. The plasma concentrations (mean $\pm$ s.e.mean) measured following intramuscular injection of drug on days 2, 3 and 4 of the study are presented in Figure 1. Peak plasma concentrations were obtained 1–2 h after administration. This was followed by an apparent monoexponential decline in concentration with a mean half-life of approximately 7 h which did not alter significantly during the course of the study (paired *t*-test $P > 0.4$). The areas under the plasma concentration-time curves for the first and last dose were also not significantly different (mean 608 ± 35 cf $568 \pm 22 \mu\text{g ml}^{-1} \text{ h}$ respectively, paired *t*-test $P > 0.05$).

Urinary recovery data are presented in Figure 2. In the 12 h following each dose the mean recovery of unchanged drug increased from 36% (± 5) (dose 1) to 51% (± 7) following the fifth dose at steady state.

Discussion

Ro13-9904 was well tolerated and produced no obvious systemic side-effects, a finding confirmed by routine haematological and biochemical testing. It remains uncertain whether the mild change in bowel action experienced by three subjects is attributed to the drug or to the change in environment on admission to hospital. Since pain had previously been reported following intramuscular administration on Ro13-9904 500 mg dissolved in 3 ml of saline, we used 1% lignocaine as diluent and minimised discomfort; local irritation was not severe but the rise in creatine phosphokinase noticed however suggests that there may be some local tissue damage at the injection site. Nevertheless two subjects commented that the injections were 'not as unpleasant as a penicillin injection'. Data from clinical trials suggests that more dilute solutions, which are isotonic, are better tolerated.

Ro13-9904 kinetics following intramuscular injection could be described in terms of a one-compartment open model. The biexponential decay observed after intravenous administration (Seddon *et al.*, 1980) was not demonstrated due to the relatively slow systemic distribution of drug. The small volume of distribution measured supports early observations of the distribution of the drug only into vascular fluids, some extravascular fluid and highly vascularised organs such as liver, kidneys and eye (Seddon *et al.*, 1980). Access to important infection sites however is not restricted. Effective drug concentrations have been measured in the cerebrospinal fluid of dogs with experimental meningitis (Marchou, Tran, Auvergnat, Massip, Armengaud & Stoeckel, 1980)

Table 1 Pharmacokinetic parameters calculated for Ro13-9904 following intramuscular injection to six normal volunteers.

Subject	Body weight (kg)	Peak plasma concentration (Cp) ($\mu\text{g ml}^{-1}$)	Day 2				Total body clearance $Q_B(i.d.)$ (lh^{-1})	Peak plasma concentration Cp ($\mu\text{g ml}^{-1}$)	Day 4			
			Time to peak Tp (h)	Half-life $T_{1/2}$ (h)	$AUC_{0-\infty}$ (i.d.) ($\mu\text{g ml}^{-1} \text{ h}$)	$V_d(i.d.)$ (l)			Time to peak Tp (h)	Half-life $T_{1/2}$ (h)	$AUC_{(24)}$ ($\mu\text{g ml}^{-1} \text{ h}$)	
1	69.8	59.2	2.0	7.1	706	7.3	0.7	75.8	2.0	6.7	630	
2	68.0	55.7	2.0	6.2	600	7.5	0.8	72.8	2.0	6.3	558	
3	80.7	51.0	1.0	8.3	660	9.1	0.8	76.1	2.0	6.0	573	
4	70.8	66.5	1.5	5.4	569	6.8	0.9	86.5	2.0	4.9	618	
5	66.2	46.2	1.5	5.6	463	8.7	1.1	68.8	1.5	5.1	483	
6	85.7	50.5	2.0	7.8	647	8.7	0.8	64.8	1.0	11.3	548	
Mean	73.5	54.9	1.7	6.7	608	8.0	0.9	74.1	1.8	6.7	568

Figure 1 Plasma concentration of Ro13-9904 (mean $\pm$ s.e.mean) in six normal subjects each receiving 2500 mg drug in five divided doses at 0 and 12 h (day 2), 0 and 12 h (day 3) and 0 h (day 4).

● day 2 (dose 1)

□ day 3 (pre- and post-dose 3)

△ day 4 (dose 5)

Figure 2 Mean (+ s.e.mean) 12 hour urinary recovery of unchanged drug following successive intramuscular doses of 500 mg Ro13-9904 to six normal subjects.

and in inflammatory blister fluid in man (Seddon *et al.*, 1980). Furthermore the drug has been shown to be effective in the treatment of meningitis (Cadoz *et al.*, 1980), gonorrhoea and urinary tract infections in man (Giamarellou *et al.*, 1980).

The observed half-life of this drug ranging from 4.9 to 11.3 h is in agreement with that recorded for the terminal phase after intravenous administration (Seddon *et al.*, 1980). It is in excess of that recorded for other similar cephalosporin antibiotics and therefore in terms of less frequent dosage, offers distinct advantages to the patient. The half-life of Ro13-9904 indicates that steady state levels will be achieved within 48 h of starting a course of treatment. The results of the present study showing equivalent areas under plasma concentration time curves after the first and last doses indicate that the pharmacokinetics of this cephalosporin do not alter during repeated administration. Steady state concentrations over a limited period therefore may be accurately predicted from data obtained after a single dose. However due to the recently observed non-

linear pharmacokinetics of this drug at higher doses (Stoeckel *et al.*, 1981) this conclusion must be restricted to the low dose used in the current study.

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