Exhibit O

S.2.4. Study No : HVT/77/14

HUMAN VOLUNTEER TRIAL TO INVESTIGATE THE ORAL ABSORPTION OF ISOMERS A AND B OF CEFUROXIME E47 ESTER

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The isomeric forms of the 1-acetoxyethyl ester of cefuroxime are easily separated by crystallization, isomer A being one-tenth as soluble in water as isomer B. In the search for a better absorbed form of cefuroxime axetil, the two isomers of the drug were given to volunteers, the hope being that the more water soluble ester (isomer B) might be much better absorbed than the 50:50 mixture of isomers.

Three volunteers received 250mg cefuroxime as isomer A and 3 as isomer B, at least 2 hours after a light breakfast. Average 24h urinary recoveries were 23.4% and 29.8% and these, taken in conjunction with the serum levels, indicated that the more soluble isomer was indeed better absorbed than the less soluble. However, neither was as well absorbed at the mixture previously given (HVT/77/4), so the second part of the experiment was modified to include 2 doses of a 50:50 mix of the crystalline isomers. The urinary recovery of the latter was 45.7% in one volunteer and 17.8% in the other. The lower absorption of drug in this experiment compared with that found in HVT/77/4 was later attributed to dose form, crystalline material being more poorly absorbed than amorphous (HVT/80/30): amorphous material had been given in the previous experiments.

One volunteer experienced a mild bowel disturbance after dosing but another had unpleasant symptoms comprising severe abdominal pains starting 3 days after dosing and associated with 12 loose stools between days 3 and 6 after dosing.

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Date: 6th July 1977

Report No: HVT/77/14

Number of Copies: 23

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Trial No: HVT/77/14

Human Volunteer Trial to Investigate the Oral Absorption of Isomers A and B of Cefuroxime E.47 Ester

SUMMARY

Six volunteers took oral doses of 250 mg cefuroxime as the E.47 ester. Five took Isomer A, five took Isomer B and two, a mixture of both. Urinary recoveries averaged 21% for Isomer A, 37% for Isomer B and 32% for the 50:50 mixture.

DATES: 24th and 31st May 1977

AIMS:

1. To assess the oral absorption of isomers A and B of cefuroxime E.47 ester and to determine if either was better absorbed than had been found previously with E.47 as a mixture.

2. To assess the incidence of gastro-intestinal side effects with the two isomers of cefuroxime E.47 ester.

TRIAL MATERIAL

250 mg doses of cefuroxime as E.47 ester isomers A and B were prepared on the morning of the study as a suspension in water containing sugar, flavouring and surfactant. The mixture contained equal quantities of each isomer. (Batch no's: isomer A, GCR 1882; isomer B, GCR 1883; Mixture, GCR 1887).

METHODS

1. Volunteers

Six male volunteers from Glaxo staff (for details see Table 1).

2. Trial Design

The trial was originally designed as a two-part, cross-over comparison of the two isomers. However, when the result of first part of the experiment was known, it was decided to give to one of each group of volunteers a 50:50 mixture of isomer A and B. Volunteers received the drug as below.

Date	Isomer A	Isomer B	A + B
24th May	Vol. 1,2,3	Vol. 4,5,6	-
31st May	Vol. 4,5	Vol. 1,2	Vol. 3,6.

3. Dosing

The dose of cefuroxime was taken orally as a suspension and washed down with a glass of orange squash.

4. Diet

The volunteers had a light breakfast no later than 1½ hours before the start of the study. Coffee was provided during the morning of the study in the Clinical Pharmacology Department, and a standard sandwich lunch, fruit and coffee were provided at 1 pm. No further food was allowed until 3 pm.

5. Blood Samples

Serum for assay was obtained from blood taken at the times indicated in Table 2.

6. Urine Samples

Urines were collected at the times indicated in Table 3.

7. Cefuroxime Assay Methods

Please see Appendix.

RESULTS

1. Serum Levels Table 2.

The average observed peak serum cefuroxime level after isomer A was 2.1 µg/ml and after isomer B was 3.3 µg/ml. The average peak serum level for the two volunteers who took A + B was 1.7 µg/ml. All these peaks occurred between 1 and 3 hours after dosing.

2. Urinary Recoveries (Table 3)

The 24-hour urinary recoveries averaged 21% for isomer A and 37% for isomer B. For the 50:50 mixture of A and B the urinary recovery averaged 32%.

3. Side Effects

One volunteer (Vol. No. 5) experienced mild loose motions after isomer B. On isomer A one volunteer (No. 3) complained of severe abdominal cramping pains starting 3 days after the trial. He had twelve loose motions between days 3 and 6 after dosing. In the second part of the experiment the same volunteer (No. 3) took a 50:50 mixture of A and B with an overall absorption of 46%; he had a milder form of gastro-intestinal upset but again was troubled by stomach pains.

Isomer B had a very distinct bitter taste whereas the taste of isomer A was easily disguised by the sugar and flavouring added.

DISCUSSION

Isomer B was better absorbed than isomer A. It was, however, disappointing that the absorption of isomer B was not better than the mixture of the isomers used in the previous human volunteer experiments (refs. HVT/77/4, HVT/77/8, HVT/77/13). The isomers' relative solubilities in water differ by a factor of 10 with isomer B being the more soluble at 0.06%.

Rat experiments (Dr D M Ryan) showed a similar order of results when the isomers were presented in saline (absorption of isomer A:9%, isomer B:19%).

The severity of the gastro-intestinal upset in volunteer 3 is worrying, particularly as this was a single and not a repeat dose study. It may well be that cefuroxime is unsuitable for oral administration unless completely absorbed.

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TABLE 1

ANTHROPOMETRY

Volunteer Number	Age (years)	Height (cms)	Weight (kg)
1	33	179	75
2	42	175	75
3	29	196	92
4	30	168	76
5	31	174	83
6	27	175	61
Average	32	178	77

Table 2

Serum Levels of Cefuroxime in Male Volunteers after Oral Dosing with Isomer A and B of the E.47 Ester

Isomer	Volunteer No.	Cefuroxime concentration (µg/ml) at hours after dose								
		½	1	2	3	4	5	6	7	8
A	1	0.8	1.9	2.2	2.3	1.7	0.5	0.4	0.3	0.2
	2	0.7	1.4	2.1	1.8	1.3	0.5	0.5	0.2	0.1
	3	1.1	2.8	3.5	2.0	0.9	0.6	0.3	0.2	0.1
	4	0.9	1.3	1.2	0.8	0.5	0.3	0.2	0.1	0.1
	5	0.7	1.9	0.9	0.6	0.3	0.2	0.1	0.1	0
B	1	1.1	1.5	2.5	1.9	1.3	0.6	0.4	0.2	0.1
	2	1.0	1.6	2.9	1.6	1.1	0.8	0.4	0.2	0.1
	4	2.8	4.5	3.1	1.6	1.0	0.3	0.2	0.1	0.1
	5	1.3	3.4	3.1	1.6	1.1	0.3	0.2	0.1	0.1
	6	1.5	2.6	3.2	3.7	1.8	0.9	0.4	0.3	0.2
A + B	3	1.7	2.3	2.0	1.1	0.7	0.4	0.3	0.1	NS
	6	1.0	1.0	1.5	1.1	1.0	0.6	0.4	0.2	NS

Peak Values Underlined

Table 3

Urinary Recoveries of Cefuroxime in Male Volunteers after Oral Dosing with Isomers A & B of the E.47 Ester.

Isomer	Volunteer No.	Urinary recovery (%) at hours after dose					
		0-2	2-4	4-6	6-12	12-24	Total Recovery %
A	1	6.9	10.0	4.0	2.0	0.3	23.2
	2	5.6	7.6	3.6	1.4	0.5	18.6
	3	13.5	10.0	2.6	2.0	0.3	28.4
	4	9.1	7.6	2.6	0.16	0.1	19.5
	5	8.9	6.5	2.2	0.72	0.2	18.5
B	1	15.1	17.9	4.12	3.4	0.4	40.9
	2	16.8	18.5	7.7	2.8	0.9	46.9
	4	20.4	10.0	2.4	0.1	0	32.9
	5	9.4	9.9	3.9	1.5	0.1	24.9
	6	9.8	15.4	3.7	3.3	0.4	31.6
A + B	3	22.4	15.0	4.9	3.0	0.4	45.7
	6	9.7	10.7	3.7	3.6	0.4	17.8

Average urinary recovery isomer A = 21%

B = 37%

A + B= 32%

APPENDIX

Assay Methods for Cefuroxime

Cefuroxime was assayed in serum and urine samples by the agar diffusion technique using Oxoid Antibiotic Medium No.2 with 0.3% sodium citrate and Bacillus subtilis 1904E spore suspension at 1% or using Factor B medium with 0.5% sodium chloride and B.subtilis MB 32 spore suspension at 1%. 1904E was used for the urine assay and both 1904E and MB 32 were used for the serum assays.

Standard concentrations of cefuroxime in the range 10µg/ml - 0.62µg/ml for 1904E and 0.5µg/ml - 0.03µg/ml for MB 32 were prepared in pH 7.0 phosphate buffer. Serum and urine samples were diluted when necessary in the same buffer.

All the assays were carried out in 12" x 12" assay plates; these were incubated for 18 hours at 37°C before zone diameters were recorded.

The assay methods give results to within ± 10%.