EVALUATION IN MAN OF FENCLOZIC ACID (I.C.I. 54,450: Myalex*), A NEW ANTI-INFLAMMATORY AGENT

I. SERUM CONCENTRATION STUDIES IN HEALTHY INDIVIDUALS AND IN PATIENTS WITH RHEUMATOID ARTHRITIS

BY

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Fenclozic acid (I.C.I. 54,450; 2-(p-chlorophenyl) thiazol-4-ylacetic acid; Myalex*) (Hepworth, Newbould, Platt, and Stacey, 1969) is one representative of a series of thiazolyl acetic acids which was active in the adjuvant-induced arthritis test in rats (Newbould, 1963) and which merited more detailed evaluation on this and other laboratory models (Newbould, 1969).

Serum concentration studies in laboratory animals (Platt, 1969) showed that, at therapeutically active doses, fenclozic acid was distributed within the "albumin space" (c. 10-15 per cent. body weight) in rats, mice, guinea-pigs, dogs, and monkeys. Furthermore, a standard dose, measured in mg./kg. by mouth in each of these species gave similar peak serum concentrations. The half-life of elimination from animal serum varied between 3 hours in monkeys and 30 to 40 hours in rats. The relationship between the activity of fenclozic acid and serum levels in the rat showed that significant anti-inflammatory activity was associated with serum levels in the range 50-100 $\mu\text{g./ml.}$ (Platt, 1969). These data therefore enabled a serum level range to be defined for preliminary clinical trials in man.

The first studies in man were made in healthy individuals to enable the serum concentrations in man to be related to those found in animals. Subsequently, the compound was tested in patients with rheumatoid arthritis to whom the nature of the experiment had been explained and whose co-operation had been invited. The latter studies comprised a short trial to compare the serum concentrations of the drug in patients with those in healthy individuals, followed by a double-blind trial in patients with rheumatoid arthritis in which

the activity of fenclozic acid was compared with that of aspirin.

The object of this first paper is to present the serum concentration data obtained in preliminary studies in healthy individuals and in patients. A subsequent paper deals with the results of the double-blind cross-over trial in patients with rheumatoid arthritis.

Methods

Serum level studies were made in ten normal subjects (nine male, one female) from the staffs of the Departments of Rheumatology and Medicine, Manchester Royal Infirmary, and the Research Department of I.C.I. Ltd., Pharmaceuticals Division.

Each subject was given a single dose of 25 mg. fenclozic acid, followed by a 50 mg. dose 12 to 24 hours later. No adverse effects were noted. Seven then received a single dose of 100 mg. fenclozic acid in tablet form. Serum samples were taken at various times after the dose for determination of the concentration of the drug. The female received a single dose of 150 mg. one week later, and six of the males received a single dose of 200 mg. Serum samples were again assayed. To complete the single-dose experiments, three of the males took a single dose of 400 mg. fenclozic acid.

Following the analysis of the single-dose experimental data, seven males undertook a repeated-dose regimen: one took nineteen doses at 25 mg. twice daily, a second took nineteen doses at 50 mg. twice daily, a third took twenty doses at 100 mg. twice daily, three took seven doses at 150 mg. twice daily, and the seventh took seven doses at 200 mg. twice daily. Serum samples were taken during and after the treatment period for drug concentration studies.

To facilitate studies in patients, fenclozic acid was administered in capsule form. To ensure that comparable absorption could be obtained from tablet and capsule formulations, three of the normal subjects

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took single doses (200 mg.) of each form. The serum levels were compared in each subject for each formulation, no significant difference being detectable.

Five patients with rheumatoid arthritis were given repeated doses of fenclozic acid to enable a comparison to be made with the results from the normal individuals. One patient received three doses of 50 mg. at 12 hourly intervals followed by twelve doses of 100 mg. twice daily, while the remaining four patients took fifteen doses of 150 mg. twice daily. Serum samples were taken during and after treatment for drug assay.

In both groups studied, all other medication was withdrawn, with the exception of chloroquine and paracetamol, neither of which interfere with the assay of fenclozic acid.

The concentration of fenclozic acid in serum was assayed by the method reported elsewhere (Platt, 1969). In addition to the drug concentration determinations, assays were also made on all the samples obtained for the activities of serum glutamic-oxalacetic transaminase (SGOT), isocitrate dehydrogenase (ICDH), and alkaline phosphatase, and for the concentrations of total protein and albumin. Routine haematology was performed on the patient samples.

Analysis of the accumulation of fenclozic acid in serum after repeated administration was made using the mathematical techniques given by Hammer and Brodie (1967), van Rossum and Toney (1968), and Platt (1969).

Results

Maximum serum concentrations in healthy individuals were observed at 3 hrs (or less) after an oral dose (Table I) and there was a linear relationship between this dose and the peak serum concentration in the dose range examined, *c.* 1 to 7 mg./kg. (Figure). An average peak level of 9.3 μ g./ml. was associated with a single dose of 1 mg./kg. body weight. The average serum half-life was 25 hrs (range 18 to 31). An accurate estimate of the volume of distribution (*V*_d) is not possible from the results given in Table I, but an approximation of the apparent *V*_d is shown in Table I, indicating that fenclozic acid is distributed at most into a volume corresponding to the albumin-space of the body. In the range of doses examined, the dose level had no effect on either the serum half-life or the apparent *V*_d of the drug.

Figure.—Relationship of dose to peak serum concentration in healthy human subjects given single oral doses of fenclozic acid.

The properties of fenclozic acid in man were essentially similar to those observed in rats, guinea-pigs, and dogs (Table II), man giving the highest serum levels dose for dose. Equilibrium dialysis experiments have shown that fenclozic acid is extensively bound to serum proteins, particularly albumin, in both animal and human serum.

TABLE II
FENCLOZIC ACID. COMPARISON OF SERUM CONCENTRATIONS AND HALF-LIVES IN MAN AND LABORATORY ANIMALS AFTER SINGLE ORAL DOSES

Species	Dose Range* (mg./kg.)	Peak Serum Concentration at a Dose of 1 mg./kg. (μ g./ml.)	Mean Serum Half-life (hrs)
Man	1.1-6.6	9.3†	25
Monkey	5-125	4.5	3
Dog	2.5-10	6.7	28
Guinea-pig	2.5-20	6.2	25
Rat	2.5-20	6.2	34
Mouse	2.5-50	5.0	<i>c.</i> 11

*Dose range in which there is a linear relationship between peak serum concentration and dose.

†In patients with rheumatoid arthritis, the average peak level is about 7.9 μ g./ml.

TABLE I
SERUM LEVELS OF FENCLOZIC ACID IN HUMAN SUBJECTS FOLLOWING A SINGLE ORAL DOSE*

No. of Subjects	Dose		Mean Serum Concentration (μ g./ml.): Hours after Dose						Mean Serum Half-life (hrs)	Mean† Apparent <i>V</i> _d (Per cent.)
	mg.	mg./kg.	3	7	24	48	72	96		
7	100	1.47±0.09	13.6±0.9	11.2±0.4	5.4±0.6	3.8±0.4	2.1±0.2	—	25±1.4	10.7±0.4
7	150-200	2.76±0.14	25.9±0.4	21.8±1.3	11.8±0.5	7.2±0.7	4.4±0.8	2.3±0.3	25±1.4	10.4±0.5
3	400	6.07±0.29	55.2±1.5	—	27.2±2.3	12.1±3.6	—	3.2±1.0	23±3.0	10.6±0.7

*mean values ± S.E.

†Apparent volume of distribution (*V*_d) derived by expressing the dose (mg./kg.) as a percentage of the serum concentration at zero time (obtained by extrapolation back to zero time of the linear semilog plot of serum concentration against time).

Repeated administration of the compound showed that fenclozic acid accumulated in human serum (Table III). The rate and extent of accumulation were in reasonable agreement with calculations based on theoretical considerations of drug accumulation, indicating that in each case a maximum plateau level would have been achieved if dosing had been maintained for a sufficient time. The calculated plateau levels for each subject are given in Table III. In two of the three cases (Subjects 6 and 7) where the plateau had been reached, there was good agreement between observed and calculated values, but in the third (Subject 5), the observed maximum of between 63 and 72 $\mu\text{g./ml.}$ was about 20 $\mu\text{g./ml.}$ higher than the calculated. A sharp rise occurred in Subject 5 between Doses 7 and 9, and it may be significant that this subject undertook strenuous physical exercise just before Dose 8. In the other four cases, insufficient doses were taken to reach the maximum levels, but with the exception of Subject 1, there was reasonable agreement between the observed levels and the calculated rate of accumulation. Subject 1 showed levels about 20 $\mu\text{g./ml.}$ higher than calculated. The reason for this is not understood, although this subject was unable for various reasons to take his doses at constant time intervals. The average half-life in five subjects was 31.4 ± 3 hrs compared with a value of 25 hrs observed in these same individuals after a single dose.

Urine collected from three of the healthy subjects showed that fenclozic acid was mainly excreted either unchanged or as an esterconjugate (Foulkes, 1969); quantitative excretion data indicated complete absorption following oral administration. No

metabolites were found in the serum of either the normal individuals or the patients at any time in these studies. No abnormal changes were observed in SGOT, ICDH, or alkaline phosphatase activities.

Accumulation of fenclozic acid in serum after repeated administration was again apparent in patients with rheumatoid arthritis (Table IV, opposite), and, as in the normal subjects, the rate and extent of this accumulation were in good agreement with theoretical calculations. An average peak serum concentration of 7.9 $\mu\text{g./ml.}$ was associated with a single dose of 1 mg./kg. in patients with arthritis, *i.e.* less than that observed in healthy individuals, but the average half-life in these five patients was 46 ± 7 hrs, *i.e.* considerably longer than in the healthy individuals.

The arthritic patients had low normal or sub-normal albumin levels (mean 3.25 g. per cent.; range 2.5 to 3.9) as well as low albumin: total protein ratios (mean 44 per cent.; range 37 to 53). No changes due to fenclozic acid administration were observed in either SGOT, ICDH, or alkaline phosphatase activities, although all five patients showed high alkaline phosphatase levels before treatment which persisted unchanged during treatment. Haematological tests were normal.

No clinical side-effects were observed in either the normal subjects or the patients.

Discussion

These studies demonstrated that the properties of fenclozic acid in serum are similar in animals and

TABLE III
FENCLOZIC ACID. SERUM LEVELS AND HALF-LIVES IN MALE SUBJECTS AFTER REPEATED ORAL ADMINISTRATION

Subject No.	Dose*		No. of Doses	Serum Concentration ($\mu\text{g./ml.}$)† after Dose No.							Serum Half-life** after Last Dose (hrs)	Calculated Plateau† (maximum) Serum Concentration ($\mu\text{g./ml.}$)
	mg.	mg./kg.		1	3	5	7	9	15	19		
1	200	3.05	7	19.8	—	63.2	94.5	—	—	—	22	80
2	150	1.71	7	17.5	40.0	—	70.0	—	—	—	34	86
3	150	2.04	7	18.4	42.5	—	76.4	—	—	—	33	88
4	150	1.92	7	19.3	44.3	—	74.9	—	—	—	40	101
5	100	1.30	20	7.6	28.3	37.7	42.6	64.7	63.4	72.1	28	50
6	50	0.65	19	7.9	14.5	17.5	19.2	20.6	22.2	21.7	—	24
7	25	0.43	19	3.8	6.4	8.0	10.1	9.9	9.2	8.9	—	9

*Doses given every 12 hours.

**Determined from observations made up to 126 hrs after the last dose.

†Peak levels 2 to 3 hrs after dose.

‡Plateau maximum concentration calculated (Methods Section) from the observed serum half-life, the average maximum concentration after one dose, and a fixed dosing interval of 12 hrs.

TABLE IV
FENCLOZIC ACID. SERUM LEVELS AND HALF-LIVES IN PATIENTS WITH RHEUMATOID ARTHRITIS
AFTER REPEATED ORAL ADMINISTRATION

Subject No.	Sex	Dose*		No. of Doses	Serum Levels ($\mu\text{g./ml.}$) after Dose No. †			Serum ‡Half-life (hrs)
		mg.	mg./kg.		1	5	15	
8**	F	50-100	0.8	15	8.9	40.6	89.0	40
9	F	150	2.32	15	20.7	67.4	126	61
10	M	150	1.75	15	11.1	46.5	62.3	43
11	M	150	2.17	15	—	44.5	58.0	27.5
12	M	150	2.16	15	—	56.5	90.5	51
Mean $\pm$ S.E.		150	2.10 $\pm$ 0.12	15	15.9	53.7 $\pm$ 5.3	84.2 $\pm$ 15.7	45.8 $\pm$ 7.1

*Doses given at 12-hr intervals.

**This patient received three doses of 50 mg. and twelve doses of 100 mg.

†Levels recorded 2 to 3 hrs after dose.

‡Half-life determined from samples taken up to 98 hrs after 15th dose.

man and that the compound is distributed within the albumin-space of the body (c. 10 to 15 per cent. body weight) and has a long half-life. After repeated administration, accumulation of the compound occurs in human serum, the rate and extent of which are predictable.

It is of interest that the compound has a greater average half-life in arthritic patients than in healthy individuals, despite a lower dose-serum level response after single doses. The five arthritic patients had low serum albumin levels in agreement with previous observations (*e.g.* Reynolds and Cluff, 1960; Bernstein and Allerhand, 1964). It is possible that the reduced serum levels of fenclozic acid in these patients after single doses were related to the low albumin levels, since Bayles (1963) has observed a similar phenomenon for salicylate levels in arthritic patients. The binding characteristics of fenclozic acid to serum albumin could also be affected in the disease state, possibly leading to an altered rate of elimination from serum, *i.e.* half-life but this has yet to be confirmed.

The results of these studies in normal subjects and patients suggested that doses of 100-200 mg. twice daily would give serum levels of fenclozic acid in man comparable with those required for significant anti-inflammatory activity in laboratory animals, *i.e.* 50 to 100 $\mu\text{g./ml.}$ The double-blind

cross-over trial against aspirin reported in our second paper was carried out, therefore, on the basis of this information.

Summary

Fenclozic acid has been administered orally to normal subjects and also to patients with rheumatoid arthritis. Serum concentrations of the drug were measured and related to results obtained in laboratory animals. Fenclozic acid was found to have a long serum half-life in man and to be distributed within the albumin-space of the body. Patients with rheumatoid arthritis showed a lower serum concentration-dose response than healthy individuals but exhibited a longer serum half-life of the drug. Repeated administration experiments enabled the rate and extent of accumulation of fenclozic acid in human serum to be assessed. Doses suitable for the preliminary evaluation of the anti-inflammatory activity of fenclozic acid in a double-blind cross-over trial against aspirin were defined from the results presented.

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L'évaluation de l'acide fenclozique, un nouvel agent anti-inflammatoire (I.C.I. 54, 450; Myalex) chez l'homme
I. Études des concentrations sériques chez les sujets sains et les malades atteints de polyarthrite rhumatoïde

RÉSUMÉ

L'acide fenclozique a été administré par voie buccale à des sujets sains et aussi à des malades atteints de polyarthrite rhumatoïde. Les concentrations sériques de ce médicament ont été évaluées et comparées aux résultats obtenus chez les animaux de laboratoire. On a trouvé que l'acide fenclozique avait une longue durée d'activité dans le sérum chez l'homme et qu'il était distribué dans l'albumine du corps. Les malades atteints de polyarthrite rhumatoïde montraient une plus basse concentration sérique par dose que les personnes saines mais présentaient une plus longue durée d'activité du médicament dans le sérum. Des administrations répétées ont permis d'évaluer le taux et l'étendue de l'accumulation de l'acide fenclozique dans le sérum humain. Des doses appropriées à l'évaluation préliminaire de l'activité anti-inflammatoire de l'acide fenclozique comparée à celle de l'aspirine pendant des expériences faites par la méthode "double-blind cross-over" ont été définies d'après les résultats présentés.

Evaluación en el hombre, de ácido fenclosico (I.C.I. 54,450; Myalex), un nuevo agente antiinflamatorio
I. Estudios de concentración de suero en individuos sanos y en pacientes con poliartritis reumatoide

SUMARIO

Se ha suministrado, por vía oral, ácido fenclosico a sujetos normales y también a pacientes con poliartritis reumatoide. Se midieron las concentraciones séricas de la droga y se relacionaron con resultados obtenidos en animales de laboratorio. Se descubrió que el ácido fenclosico tenía un periodo largo en el suero en el hombre y que se distribuía en la albúmina del cuerpo. Los pacientes con poliartritis reumatoide mostraron una reacción más débil que los individuos sanos, pero revelaron un período sérico más largo de la droga. Repetidos experimentos permitieron determinar la tasa y el grado de acumulación de ácido fenclosico en el suero humano. Las dosis adecuadas para la evaluación preliminar de la actividad antiinflamatoria del ácido fenclosico en una prueba cruzada dobleciego con aspirina se determinaron a base de los resultados presentados.