PRELIMINARY STUDIES OF ABSORPTION AND EXCRETION OF BENOXAPROFEN IN MAN

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- 1 Benoxaprofen is a new acidic anti-inflammatory compound which was well absorbed after oral administration to man.
- 2 Single doses of 100, 200 and 400 mg produced mean peak concentrations in the plasma of 13.0, 33.5 and 45.3 $\mu\text{g/ml}$ respectively, and the plasma half-life of the compound was between 30 and 35 hours.
- 3 Multiple dosing with 25 and 50 mg every 24 h achieved an equilibrium concentration in the plasma after 6–8 days, while dosing with 100 mg every 12 h enabled equilibrium to be reached in 3–6 days. Plasma concentrations between 35 and 45 $\mu\text{g/ml}$ were achieved by giving 100 mg doses every 12 hours.
- 4 Absorption of benoxaprofen was delayed when the drug was given with food, but the total amount absorbed remained the same.
- 5 The effect of milling the material to small particle size (19 μm) was to increase the rate of absorption compared to that of unmilled material (58 μm).
- 6 Benoxaprofen was well tolerated by healthy male subjects in the doses given.

Introduction

Benoxaprofen (Figure 1) is a novel acidic compound (Dunwell, Evans, Hicks, Cashin & Kitchen, 1975) exhibiting notable anti-inflammatory, analgesic and antipyretic activity in animals (Cashin, Dawson & Kitchen, 1977). Single-dose studies in several animal species have shown that the compound is well absorbed after oral administration (Chatfield & Green, 1977). In the adjuvant arthritis test conducted in rats, it has been shown that with steady-state plasma levels of 5 $\mu\text{g/ml}$ there is a 30% reduction in secondary lesions as measured by paw volume, and with plasma levels of 35 $\mu\text{g/ml}$ the reduction is 70% (Chatfield, Cashin, Kitchen & Green, 1977). Therefore initial studies in human volunteers were planned with the object of establishing the oral dose of benoxaprofen which would produce plasma concentrations in the range 5–35 $\mu\text{g/ml}$ and to provide other data useful in the design of the initial clinical trials.

The studies described here involve the oral administration of benoxaprofen to healthy human subjects in single doses of 100, 200 and 400 mg, and in multiple doses of 25 and 50 mg once daily and 100 mg twice daily. Further single dose studies were carried out to compare the absorption of benoxaprofen in fasted and non-fasted subjects and to investigate the effect of particle size on absorption.

Methods

Subjects

The seventeen subjects selected for these studies were fully informed male volunteers shown to be normal and healthy by clinical, haematological and biochemical examinations conducted beforehand. Their ages lay in the range 21–55 years and their body weights in the range 51–90 kilograms. Six of the subjects took part in more than one study.

Formulation

In the initial single-dose study benoxaprofen (100 mg) was unmilled and had a mean particle size of 23.5 μm (all <125 μm). In the multiple dose studies at 25 and 50 mg the mean particle size was 58 μm (all <180 μm) and this material was shown in a separate experiment in four volunteers to be bioequivalent to the 23.5 μm batch (Smith & Chatfield, unpublished observations). Benoxaprofen was administered in these studies as a 20% w/w mixture with maize starch in hard gelatin size two capsules.

The studies of 200 and 400 mg single doses and 100 mg multiple doses were carried out using material which had been milled to a mean particle size of 19 μm (all <125 μm). The compound was

Figure 1 Structure of benoxapofen (2-(4-chlorophenyl)- α -methyl-5-benzoxazole acetic acid).

administered in gelatin capsules containing benoxapofen (100 mg) at 30% w/w with starch diluent.

Single-dose studies

Single doses of benoxapofen (100 mg) were given by mouth to eight subjects at 09.00 h after an overnight fast. Four other subjects received single doses of 200 mg and 400 milligrams. The subjects (all non-smokers) swallowed the capsules with 50 ml water whilst standing. They remained either ambulant or seated for at least 12 h after receiving the capsules.

Samples of venous blood and urine were collected immediately before administration of the drug, and further blood samples were collected at varying intervals for periods ranging from 24–168 h thereafter. Blood samples were collected into lithium-heparin tubes, and centrifuged. The plasma was then separated into plain containers and frozen at -20°C to await assay.

Urine was collected for periods ranging from 48 h–96 h after dosing in different subjects. These samples were also frozen while awaiting assay.

On each occasion, a light breakfast of orange juice, rolls, butter, marmalade and coffee was served to the subjects about 1.5 h after dosing, and luncheon was taken normally at about 3.5 h after dosing.

Effect of food

The effect of food on the absorption of benoxapofen was studied separately; two subjects who had

previously received benoxapofen (100 mg) in the fasted condition were selected. After an overnight fast, both subjects were given one round of ham sandwiches and a cup of coffee at about 10.00 h and immediately afterwards took benoxapofen (100 mg). Luncheon was taken at the normal time between 2 and 3 h later. The study was carried out in the same manner as the other single-dose studies; plasma samples were collected before and at intervals up to 24 h after dosing, and assayed for benoxapofen levels. These were compared with the levels obtained in the fasted state.

Effect of particle size

The effect on absorption of milling the material was studied by giving single doses of 100 mg of each of two formulations to a group of seven subjects, with an interval of fourteen days between doses. The unmilled material had a mean particle size of $58\text{ }\mu\text{m}$ and the milled material had a mean particle size of $19\text{ }\mu\text{m}$. Capsules contained benoxapofen (100 mg) at 36% w/w with starch diluent. In other respects, the study was conducted in the same way as the earlier ones.

Multiple-dose studies

Benoxapofen was given at about 09.00 h to three groups of four subjects in doses of 25 mg once daily, 50 mg once daily and 100 mg twice daily, respectively. Dosage was continued for 11 days. Food was freely available at customary times, with luncheon being taken about 3 h after the morning dose.

Plasma samples were collected 6 h and 24 h after the morning dose to provide estimates of the peak and trough levels of benoxapofen. Urine collection was carried out for a 24-h period on one or two occasions during each study.

At the conclusion of 11 days dosing, the rate of decline in benoxapofen plasma concentrations was observed over the ensuing 96 hours.

Table 1 Urinary recovery of benoxapofen from individual subjects after single doses of 100 mg

Period of urine collection (h)	Urinary recovery (% dose)		
	Unchanged benoxapofen	Glucuronide conjugate	Total
0–48	5.9	13.0	18.9
	4.1	21.1	25.2
0–72	3.9	20.9	24.8
	3.1	21.5	24.6
	6.6	23.4	30.0
0–96	5.1	48.8	53.9
	5.7	41.0	46.7
	4.2	51.0	55.2

Assay of benoxaprofen

The levels of benoxaprofen in plasma and urine samples were assayed by gas chromatography using flame ionization for detection of the methyl ester derivative. The method will be described in detail elsewhere (Chatfield & Woodage, 1977), and has a sensitivity down to 0.1 µg/ml.

Safety monitoring

The clinical, haematological and biochemical examinations conducted before testing were repeated after each dose in the case of the single dose studies, and in the multiple dose studies were repeated additionally at intervals of 2 or 3 days during the period of drug administration. The clinical examination comprised a physical examination, electrocardiogram and Vitalograph. The haematological tests were erythrocyte, leucocyte and thrombocyte counts, haemoglobin, haematocrit, mean corpuscular volume, platelet factor 3 availability, thrombin time, partial thromboplastin time, and bleeding time. Platelet aggregation tests were also performed in association with the higher doses. The biochemical tests were total protein, albumin, urea, cholesterol, total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, sodium and potassium. Urine samples were tested for specific gravity, reaction, and the presence of protein and sugar, and were also examined microscopically.

Results

Single-dose studies

Assay of the plasma samples taken from subjects receiving single doses of benoxaprofen at 100, 200 and 400 mg demonstrated mean peak concentrations of the unchanged compound of 13.0, 33.5 and

Figure 2 Plasma concentrations of benoxaprofen after single oral doses. Results are shown as the mean $\pm$ s.e. mean for eight subjects at 100 mg (●) and four subjects at 200 mg (○) and 400 mg (■).

45.3 µg/ml respectively (Figure 2). Peak concentrations occurred 2–4 h after dosing. After reaching a peak, the mean plasma concentrations fell slowly. The mean plasma half-life was found to be long, in most subjects between 30–35 h, although a considerably longer half-life was recorded in some individuals (Chatfield, Tarrant, Smith & Speirs, 1977). The related areas under the 0–72 h mean plasma level curves were 397, 940 and 1480 µg h ml⁻¹, indicating a reasonably linear dose-response in absorption over this dosage range.

After the 100-mg dose benoxaprofen was recovered from the urine chiefly as the glucuronide conjugate, though a small fraction of the administered dose was found in the urine in the unchanged form (Table 1). Considerable inter-subject variation in total recovery was observed (range 19 to 55% of the dose) but total collection periods were not identical for each subject.

Table 2 Effect of food on absorption of benoxaprofen

Time (h)	Plasma concentration of benoxaprofen (µg/ml)			
	Subject A		Subject B	
	Fasted	Non-fasted	Fasted	Non-fasted
2	14.0	6.7	9.4	3.7
3	13.1	8.3	17.0	10.7
4	11.8	10.7	13.8	9.9
6	11.2	12.7	12.8	15.7
24	5.9	4.9	6.8	8.5
Area under curve (0–24 h) by trapezoidal rule (µg h ml ⁻¹)	216.9	199.5	241.0	264.6

Effect of food

The results (Table 2) show that the presence of food in the stomach decreased the absorption rate of benoxaprofen. The peak level appeared to be reached about 6 h after dosing, compared with 2 to 3 h after dosing on an empty stomach. The levels at 6 h after dosing were higher when the drug was taken after food. In both subjects the area under the linear curve (0–24 h) showed individual variation but was approximately the same whether fasted or not, indicating that the total absorption of the drug was similar in both circumstances.

Effect of particle size

The results of this study (Figure 3) indicate that, at 1 and 2 h after dosing, the milled (19 μm) material produced higher mean plasma levels (10.4 and 12.9 $\mu\text{g/ml}$) than those produced by the unmilled (58 μm) material (2.8 $\mu\text{g/ml}$ and 8.1 $\mu\text{g/ml}$ respectively). These differences were significant at the 99% confidence limits by Student's *t*-test. Neither the differences in mean levels at 3, 4, 6 and 24 h after dosing, nor the difference in time to mean peak level (2.4 h and 3.2 h respectively) were significant. The area under the mean linear 0–24 h plasma concentration curve for the unmilled material was about 10% lower than that for the milled material suggesting that in this experiment slightly less of the unmilled material was absorbed. However, the plasma levels obtained in this experiment for the unmilled material were lower than observed in the previous 100 mg study (Figure 2). The major effect of milling from a mean particle size of 58 μm down to 19 μm is therefore to increase the rate of absorption. The amount absorbed remains about the same and is considered to be essentially complete for both formulations.

Figure 3 Plasma concentrations (mean $\pm$ s.e. mean, $n=7$) of benoxaprofen after administration of 100 mg doses of milled material (●), mean particle size 19 μm , and unmilled material (○), mean particle size 58 μm .

Multiple-dose studies

The results of the single-dose studies suggested that plasma levels in the range 5 to 35 $\mu\text{g/ml}$ could be achieved by administration of benoxaprofen once

Figure 4 Plasma levels (mean $\pm$ s.e. mean, $n=4$) of benoxaprofen at 6 h (○) and 24 h (●) after multiple dosing of 25 mg every 24 h (at arrow). The dotted line joins the plasma levels observed after the final dose.

Figure 5 Plasma levels (mean $\pm$ s.e. mean, $n=4$) of benoxaprofen at 6 h (○) and 24 h (●) after multiple dosing of 50 mg every 24 h (at arrow).

Figure 6 Plasma levels (mean $\pm$ s.e. mean, $n=4$) of benoxaprofen after multiple dosing of 100 mg every 12 h (at arrow). The continuous lines join trough pre-dose levels to the 6 h levels achieved after each dose and the dotted line joins the levels observed after the penultimate dose.

daily, and the first two multiple-dose studies were conducted at this dose frequency.

A daily dose of 25 mg produced a steady state after 6–8 days with mean plasma concentrations ranging between a level at 6 h of 5.8–6.7 $\mu\text{g/ml}$ and a trough of 4–5 $\mu\text{g/ml}$ (Figure 4). Daily doses of 50 mg produced a steady state after 4–6 days with mean 6 h concentrations of 10.2–11.8 $\mu\text{g/ml}$ and mean trough concentrations ranging between 6.7 and 7.4 $\mu\text{g/ml}$ thereafter until cessation of dosing (Figure 5).

The rate of decline in benoxaprofen plasma concentration over the ensuing 96 h was very similar to the decline in plasma concentration following single doses.

In the third multiple-dose study, benoxaprofen (100 mg) was given orally to four subjects at intervals of 12 hours. In this study, the steady state was reached after 3 days in two of the subjects, but only after 6 days in the other two. The mean 6 h plasma concentration was 43.2 $\mu\text{g/ml}$ and the mean trough level 37 $\mu\text{g/ml}$. After stopping the drug, the plasma concentrations declined in a manner similar to that observed in the other studies (Figure 6).

The urinary recovery of benoxaprofen in both free and conjugated form in these studies was similar to that observed in the single dose studies and showed the same degree of inter-subject variation. After the tenth dose of 25 mg a mean 24 h total recovery of 24% (range 18–35%) of the daily dose was obtained. Corresponding recoveries after the fifth and eighth doses at 50 mg were 37% (range 24–53%) and 30% (23–35%) respectively. After the fifteenth dose of 100 mg, a 24 h recovery was similar, at 44% (range 33–53%) of the total daily intake (200 mg).

Safety monitoring

No abnormalities of any kind were detected by clinical examination of any of the subjects admitted to the studies.

After the 400-mg dose, all four subjects showed some suppression of collagen-induced platelet aggregation during part of the observation period. In three subjects, aggregation was suppressed at 2 h and 168 h after dosing. In the fourth subject, aggregation was suppressed at 2 h but was normal at 168 hours.

Discussion

Benoxaprofen has demonstrated good activity in the adjuvant arthritis test in rats at dose levels which produced plasma concentrations between 5 and 35 $\mu\text{g/ml}$. It was therefore reasonable for the achievement of this range of concentrations to be set as a target for the initial administration of benoxaprofen to man, and thereby provide a rational basis for the dosage recommendations in the early clinical trials.

The results of the studies described here have shown that benoxaprofen was well tolerated by healthy human subjects given single doses up to 400 mg, and 100 mg every 12 h for up to 11 days. A mean plasma level greater than 5 $\mu\text{g/ml}$ which was maintained for 24 h was achieved with single doses of 100 mg, 200 mg and 400 mg. However, to obtain a mean peak level above 35 $\mu\text{g/ml}$ a single dose of 400 mg was required, and 12 h after this dose the mean plasma level of benoxaprofen was still in the region of 35 $\mu\text{g/ml}$.

The results of the multiple-dose studies have shown that steady state plasma concentrations can be maintained around the 5 $\mu\text{g/ml}$ level by single daily doses of 25 mg or 50 mg and that a dose of 100 mg given at intervals of 12 h produces a steady state level substantially higher than 35 $\mu\text{g/ml}$. The daily dose and the frequency of administration appeared to affect the number of days required to reach a steady state, but the long half-life of benoxaprofen in human plasma suggests that once a steady state has been reached, it should be possible to maintain plasma levels by dosage only once or twice daily. In the urine, benoxaprofen is excreted mainly as the glucuronide conjugate, only a small quantity appearing as unchanged drug.

The absorption of drugs from the gastro-intestinal tract is a complicated process which clinicians recognize as a possible area of therapeutic failure. Individual patient characteristics such as the rate of gastric emptying, pH of the stomach and intestine and the metabolism of the drug by intestinal flora may be as important as the physicochemical characteristics of the drug such as the dissociation constant (pK_a), lipid

solubility and particle size. The role of these factors has been reviewed recently (Prescott, 1974; Lant & Wilson, 1975). At this stage in the development of benoxaprofen we have considered only two of these factors, one an individual characteristic and one a physicochemical characteristic.

The presence of food in the stomach reduces the rate of gastric emptying and slows the rate of absorption of benoxaprofen, the attainment of peak plasma level taking almost twice as long as in the fasted state. The area under the linear curve shows individual variation but suggests that over a period of 24 h the total absorption of benoxaprofen in the fasted and non-fasted subject is approximately the same. The presentation of benoxaprofen as milled material seemed to have advantages in producing higher peak levels more rapidly than the unmilled material. However, the differences in particle size used throughout these studies did not appear to significantly affect the total amount of benoxaprofen absorbed.

Suppression of collagen-induced platelet aggregation was observed in subjects who had received single doses of 400 mg of the drug. This observation suggests that at this large dose, benoxaprofen has an effect on the platelet release reaction, which lasts for the life of the platelet. Similar effects have been attributed to aspirin; other acidic anti-inflammatory drugs such as fenoprofen and

indomethacin have been reported as having reversible effects on the platelet release reaction (Herrmann, Marshall, Crowe, Frank, Marlett & Laceyfield, 1972; O'Brien, Finch & Clark, 1970).

In conclusion, these preliminary studies have shown that benoxaprofen was well absorbed after oral administration to man and that plasma concentrations in the range of 35–45 µg/ml were achieved and maintained by a dose of 100 mg given at twelve-hourly intervals. The drug was well tolerated by healthy male subjects who received this dose for up to eleven days. While the therapeutic efficacy and safety of benoxaprofen await critical appraisal in clinical use, the results from these present studies suggest that 100 mg of milled material given twice daily would be an appropriate dose for use in initial clinical trials. The long half-life of benoxaprofen indicates a potential for the once daily dosage schedule that rheumatic patients and their practitioners considered to be important (Wright & Hopkins, 1976) and may offer a significant advantage in this field of therapy.

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