# PRELIMINARY COMMUNICATIONS

## Cefoxitin, a New Semi-synthetic Cephamycin: An In-vitro and In-vivo Comparison with Cephalothin

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*British Medical Journal*, 1973, 4, 653–655


### Summary

**The activity of cefoxitin was compared with that of cephalothin against 229 bacterial strains. Cefoxitin was more active against most Gram-negative strains, notably against indole-producing Proteus spp., which are usually resistant to the cephalosporins. Cefoxitin was not susceptible to any significant extent to degradation by β-lactamases produced by Gram-negative organisms. Against Gram-positive organisms, however, cefoxitin was considerably less active than cephalothin, but minimum inhibitory concentrations for Staphylococcus aureus were well within therapeutically attainable blood levels.**

**Pharmacokinetic studies in 18 volunteers showed a higher and longer sustained antibiotic activity in serum and urine after injections of cefoxitin than after equal doses of cephalothin. Urinary recovery of cefoxitin activity was also much higher than that of cephalothin. No evidence of toxicity due to cefoxitin was found. Cefoxitin was slightly less painful after intramuscular injection than cephalothin.**


### Introduction

The cephalosporins are superior to naturally-occurring penicillins in being more stable to staphylococcal β-lactamases.

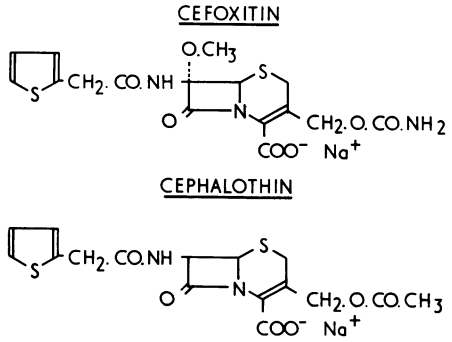


FIG. 1—Chemical formulae of cefoxitin and cephalothin. Note presence of α-methoxy-group in the 7-position in the cefoxitin molecule (a group shared by all cephamycins) and substitution of a carbamate group in the place of acetoxy group.

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However, cephalosporins are labile to the β-lactamases of many Gram-negative organisms. A new family of natural products, the cephamycins (Nagarajan *et al.*, 1971; Stapley *et al.*, 1972), offers hope of overcoming this latter handicap. Early reports indicated that cephamycin C was much less easily degraded by β-lactamases from members of the enterobacteriaceae than were cephalosporins (Daoust *et al.*, 1973). As cephamycin C is a close analogue of cephalosporin C, it was only to be expected that chemically-modified semi-synthetic cephamycins would be made, and that these may have superior antimicrobial properties. The first such compound made available for independent investigation was cefoxitin (fig. 1), a compound precisely analogous to cephalothin (Karady *et al.*, 1972). We have, therefore, studied both the antimicrobial activity and the pharmacokinetics of this compound in comparison with cephalothin, and report the results.

### Materials and Methods

*Antibiotics.*—The sodium salt of cefoxitin and of cephalothin (Keflin) were kindly provided by Dr. C. M. Martin.

*Bacterial Strains.*—Most of the strains used had been freshly isolated in the routine laboratories of this department within the past six months. Eighteen-hour cultures were used, except in the case of *Bacteroides fragilis*, for which 42-hour cultures in cooked meat medium were used.

*Determination of Minimum Inhibitory Concentration (M.I.C.).*—Serial doubling dilutions of antibiotics were made in Difco brain heart infusion agar, with 4% lysed horse blood added when required. Each plate was streaked with a $10^{-2}$ dilution of an 18-hour culture of 10 different strains, and incubated at 37°C for 18 hours (anaerobic organisms were incubated for 42 hours at 37°C with the BBL GasPak system). M.I.C. was taken as the lowest concentration of antibiotic which suppressed growth completely.

*Antibiotic Destruction.*—This was investigated qualitatively by incubating suspensions of washed organisms (concentrated fourfold from 18-hour cultures) with 200 μg/ml of cefoxitin or cephalothin in phosphate-citrate buffer, pH 7, for five hours at 37°C. Residual antibiotic was determined by the microbiological assay described below.

*Clinical Pharmacology.*—Cefoxitin and cephalothin were given to 18 male human volunteers, aged from 21 to 37 years. Physical examination and laboratory tests before the study did not show evidence of renal, hepatic, cardiopulmonary, haematological, or any other systemic disease. Subjects with a history of allergy to penicillin were excluded. The volunteers were randomly divided into two groups. One group received three injections of cephalothin in weekly intervals, while the other received cefoxitin in the same manner. The three dosages given to each volunteer were: 0·5 g and 1·0 given intramuscularly and 2·0 g given intravenously as a bolus injection lasting three minutes, the order of administration being allocated randomly. Ten serial venepunctures and seven urine collections were made before and up to 12 hours after each intramuscular injection. The schedule was slightly different after intravenous dosage, 11 venepunctures and six urine collections being taken. The precise timings of these 54 profiles are given in the Results section. The duration and intensity of pain at the site of the injection were recorded. Pain was classified subjectively as follows: 0 = no pain, 1 = mild, 2 = moderate, and 3 = severe pain. Toxicity studies made before and two days after each dose included white blood count, packed cell volume, haemoglobin, platelet count, blood urea,

serum aspartate aminotransferase, alkaline phosphatase, total and conjugated bilirubin, as well as urine pH, specific gravity, glucose, protein, and microscopy. All subjects were closely observed over the whole study for the development of side effects.

*Antibiotic Assays.*—The antibiotic activity of each specimen was estimated by microbiological assay: 100-ml amounts of brain heart infusion agar (Difco) containing 1 ml of an overnight culture of *Staphylococcus aureus* MB-2786 (kindly supplied by Dr. C. M. Martin) were poured into plastic plates 23·5 cm square. Standards (50, 25, 12·5, and 6·3 μg/ml) and specimens (suitably diluted when necessary) were taken up on to filter paper discs 6 mm in diameter; each disc was found to imbibe 20 μl. When assaying sera pooled human plasma was used as diluent, and Sørensen's phosphate buffer, pH 6, was used when urine specimens were assayed. Discs were set 42 to a plate so that each plate contained one complete series of specimens as well as standards in triplicate. Specimens were set in triplicate, and in many cases two dilutions of each specimen were set, each in triplicate. Plates were incubated at 37°C overnight, and zone diameters were read using calibrated calipers or a magnifying projection system.

## Results

*Comparative Antimicrobial Activities of Cephalothin and Cefoxitin.*—M.I.C. data obtained with 200 bacterial strains (not including pseudomonads) are given in fig. 2. Certain of these data were processed and are shown in table I. When comparing the two antibiotics it was against the indole-producing *Proteus* strains (5 *Pr. vulgaris*, 5 *Pr. morganii*, 2 *Pr. rettgeri*) that cefoxitin showed its highest degree of superiority. Only one strain out of the 12 tested was resistant to cefoxitin, and conversely only one strain of the 12 was sensitive to cephalothin. Against *Pr. mirabilis* the two compounds were virtually equipotent, against *Escherichia coli*, *B. fragilis*, and *Acinetobacter* spp. cefoxitin was slightly superior to cephalothin, while against *Klebsiella* spp. cefoxitin was consistently superior. Twenty-three strains of *Enterobacter* spp. were tested (18 *E. cloacae*, 3 *E. aerogenes*, 2 *E. hafnia*)—five were sensitive to cephalothin and 10 to cefoxitin. All 29 *Pseudomonas aeruginosa* strains were highly resistant to both antibiotics (M.I.C. > 500 μg/ml). Against Gram-positive strains cephalothin was superior by a factor of about tenfold. Nevertheless, the highest M.I.C. for the staphylococcal strains (5 μg/ml for cefoxitin) was considerably less than the peak blood level after the smallest dose (0·5 g) of cefoxitin administered (see below). Anaerobiosis, variation in inoculum size of up to one-million-fold and changes in medium composition and pH (between 5 and 8) had only small effects on the biological activities of both antibiotics.

*Susceptibility to β-lactamase Degradation.*—All 17 strains belonging to the enterobacteriaceae which were resistant to cefoxitin were tested for their ability to destroy the antibiotic.

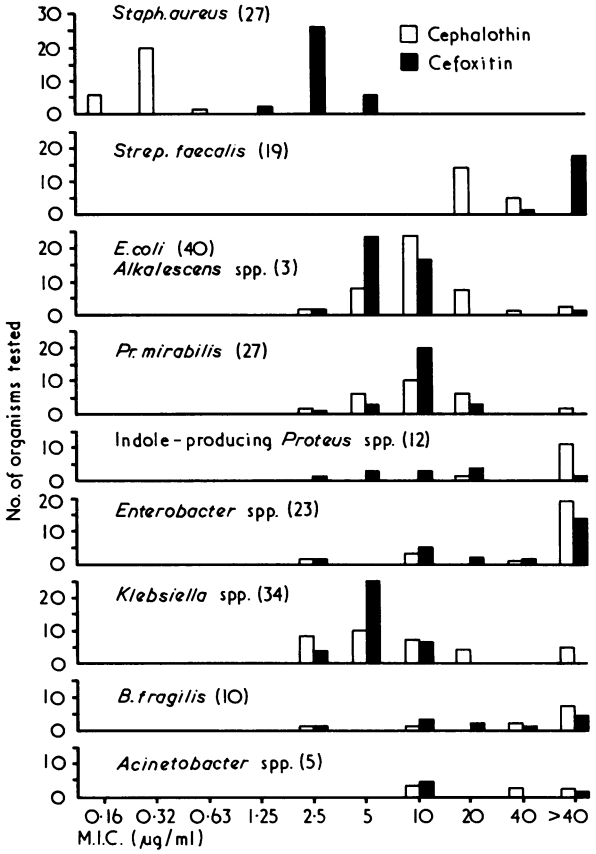


FIG. 2—Minimal inhibitory concentrations (M.I.C.) of cefoxitin and cephalothin. Figures in parentheses are total numbers of strains tested from each species.

Destruction was observed in only one case, with a strain of *E. aerogenes*, and this was only after β-lactamase had been induced by growth in the presence of cefoxitin. In contrast, all out of 26 cephalothin-resistant strains tested destroyed cephalothin to a greater or less extent. None of the strains of the *Ps. aeruginosa* or *Streptococcus faecalis* (which were highly resistant) caused any destruction of cefoxitin.

*Pharmacokinetics.*—Levels of antibiotic activity in serum after administration of cefoxitin were considerably higher and longer sustained than after cephalothin (table II). Biological terminal half life of cephalothin-like activity was 25 minutes, while that of cefoxitin was 45 minutes, as calculated from data in table II. Urine antibiotic activity was also higher in the subjects that received cefoxitin (table III), though both antibiotics produced very high activity levels in urine. However, in the last urine collections (6-12 hours after intramuscular and 4-12 hours after intravenous injection) in 22 (41%) of the cephalothin profiles the antibiotic activity was below 5 μg/ml and in 18 (33%) it was below 2 μg/ml, while in all cefoxitin

TABLE I—*Comparative In-vitro Activities of Cephalothin (CPT) and Cefoxitin (CFX)*

| Organism | No. of Strains Tested | Percentage of Resistant Strains (Resistant = M.I.C. >40 μg/ml) | | M.I.C. (μg/ml) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Median | | Mode | | C50 | |
| | | CPT | CFX | CPT | CFX | CPT | CFX | CPT | CFX |
| *Staphylococcus aureus* | 27 | 0 | 0 | 0·32 | 2·5 | 0·32 | 2·5 | 0·2 | 1·85 |
| *Streptococcus faecalis* | 19 | 0 | 94·7 | 20 | >160 | 20 | >160 | 16 | >160 |
| *Escherichia coli* (40) and *Alkalescens* spp. (3) | 43 | 4·6 | 2·3 | 10 | 5 | 10 | 5 | 7·1 | 4·5 |
| *Proteus mirabilis* | 27 | 0 | 0 | 10 | 10 | 10 | 10 | 6·7 | 7 |
| Indole-producing *Proteus* spp. | 12 | 91·7 | 8·3 | >40 | 10 | >40 | 20 | >40 | 8 |
| *Klebsiella* spp. | 34 | 14·7 | 0 | 5 | 5 | 5 | 5 | 4·6 | 3·6 |
| *Bacteroides fragilis* | 10 | 60 | 30 | 80 | 20 | 80 | 80 | 46 | 14 |
| *Acinetobacter* spp. | 5 | 40 | 20 | 10 | 10 | 10 | 10 | 9 | 6·6 |

These values have been calculated from data given in fig. 2. Median = Central value. Mode = Single most commonly occurring value. C50 = Concentration of antibiotic which inhibits 50% of the strains (determined graphically). M.I.C. = Minimum inhibitory concentration.

TABLE II—*Mean Serum Concentrations (μg/ml) of Cefoxitin (CFX) and Cephalothin (CPT)-like Activity after Three Injections each to 18 Volunteers (54 Profiles)*

| Time (min) | 0·5 g Intramuscular | | 1·0 g Intramuscular | | Time (min) | 2·0 g Intravenous | |
|---|---|---|---|---|---|---|---|
| | CFX | CPT | CFX | CPT | | CFX | CPT |
| 0 | — | — | — | — | 0 | — | — |
| 10 | 10·2 | 5·99 | 19·4 | 20·8 | 5 | 222·6 | 161·4 |
| 20 | 10·9 | 6·3 | 22·5 | 20·3 | 10 | 141·9 | 105·4 |
| 30 | 10·3 | 5·2 | 20·5 | 16·9 | 20 | 85·4 | 53·2 |
| 45 | 9·1 | 4·4 | 18·4 | 13·5 | 40 | 38·9 | 17·8 |
| 60 | 7·4 | 3·46 | 15·2 | 11·0 | 60 | 24·6 | 8·3 |
| 120 | 3·5 | 1·45 | 6·4 | 3·1 | 90 | 13·8 | 3·2 |
| 180 | 1·0 | — | 2·9 | 0·75 | 120 | 8·4 | 0·97 |
| 240 | — | — | 0·39 | — | 180 | 3·4 | — |
| 360 | — | — | — | — | 240 | — | — |
| | | | | | 360 | — | — |

TABLE III—*Mean Concentration (μg/ml) and Total Recovery of Cefoxitin (CFX) and Cephalothin (CPT)-like Activity in Urine after Three Injections each to 18 Volunteers (54 Profiles)*

| Collection Period (hours) | 0·5 g Intramuscular | | 1·0 g Intramuscular | | Collection Period (hours) | 2·0 g Intravenous | |
|---|---|---|---|---|---|---|---|
| | CFX | CPT | CFX | CPT | | CFX | CPT |
| -1–0 | — | — | — | — | -1–0 | — | — |
| 0–1 | 3,612 | 1,852 | 7,119 | 5,019 | 0–1 | 23,733 | 8,031 |
| 1–2 | 1,483 | 731 | 3,369 | 2,276 | 1–2 | 5,800 | 725 |
| 2–3 | 997 | 565 | 1,363 | 855 | 2–3 | 2,311 | 297 |
| 3–4 | 373 | 296 | 901 | 429 | 3–4 | 911 | 150 |
| 4–6 | 145 | 79·5 | 450 | 159 | 4–12 | 119 | 14·1 |
| 6–12 | 22·0 | 12·6 | 45·6 | 26·6 | | | |
| Total recovery (% of dose) | 87·1 | 52·7 | 90·1 | 55·5 | | 99 | 54·3 |

TABLE IV—*Mean Intensity and Duration of Pain (± S.D.) after Intramuscular Injections of Cefoxitin and Cephalothin in 18 Volunteers*

| | Cefoxitin | | Cephalothin | |
|---|---|---|---|---|
| | 0·5 g | 1 g | 0·5 g | 1 g |
| Mean intensity* .. | 1.6 (± 0·52) | 2·0 (± 0·5) | 1·8 (± 0·44) | 2·4 (± 0·51) |
| Mean duration (min) .. | 18 (± 5·00) | 22 (± 5·59) | 22 (± 3·63) | 24 (± 4·63) |

*Intensity of pain was graded after each injection as follows: 0 = no pain, 1 = mild pain, 2 = moderate pain, 3 = severe pain.

profiles an antibiotic activity of more than 5 μg/ml was found in all collections. Recovery of antibiotic activity in urine was also higher in the case of cefoxitin (table III). Percentages were of the order of 90%, while recovery of cephalothin-like activity was of the order of 55%.

*Toxicity Studies.*—Both drugs were non-toxic. One volunteer developed a mild leukopenia after the first injection of cephalothin (white blood count 3,200/mm³ with 1,984/mm³ polymorphs) but seven days later haematological findings were normal. There was no other clinical or laboratory evidence of toxicity in any other volunteer.

*Pain.*—Intramuscular injection of cefoxitin was slightly less painful than equal doses of cephalothin and the pain lasted marginally less (table IV). Intensity and duration of pain were unrelated to antibiotic levels obtained after the corresponding injections.

## Discussion

The single most outstanding property of cefoxitin is its remarkable resistance to degradation by β-lactamases. This does not imply that it will be active against all β-lactamase-producing organisims because of problems of intrinsic resistance. However, our findings (table I) indicate that cefoxitin has a broader antimicrobial spectrum than is shown by any other β-lactam antibiotic, either established or in the experimental stage. Nevertheless, it should be noted that even cefoxitin has a negligible activity against *Ps. aeruginosa* and *St. faecalis* and a poor one against *Enterobacter* spp. These organisms must therefore owe their resistance to an intrinsic mechanism. Further, against cephalothin-sensitive Gram-negative strains cefoxitin was usually more active than cephalothin.

The blood levels obtained after administration of cefoxitin were higher and more prolonged than those after cephalothin (table II), as were the urine levels (table III), and the terminal serum half life of cefoxitin was almost double that of cephalothin. These latter findings may at least in part be due to the fact that whereas cephalothin is deacetylated in vivo (Lee *et al.*, 1963), cefoxitin appears to be excreted virtually unchanged in man (Sonneville *et al.*, 1973). It should be pointed out that the cephalosporins which have more favourable pharmacokinetic features (such as cephanone) lack the wide and effective antimicrobial activity of cefoxitin (Meyers *et al.*, 1972; Neu and Winshell, 1973).

Like cephalothin, cefoxitin was non-toxic. The neutropenia in a volunteer receiving cephalothin may or may not have been drug related.

In conclusion, the good pharmacokinetic properties of cefoxitin together with the lower M.I.C. for most Gram-negative organisms and lack of toxicity seem very promising. These findings are sufficiently encouraging to justify clinical trials in order to define its place in chemotherapy.

We are grateful to Dr. C. M. Martin and Dr. A. Andrew, of Merck, Sharp & Dohme, for useful discussions. We also thank Merck, Sharp & Dohme for extending many facilities.

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