Cephaloglycin and Its Biologically Active Metabolite Desacetylcephaloglycin

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Chromatographic studies and microbiological assays show that, after oral administration, cephaloglycin is partially converted in man to a biologically active metabolite desacetylcephaloglycin. The antibacterial activity of this metabolite compared to that of cephaloglycin is equivalent against gram-positive organisms but is lower against gram-negative bacilli. Successful therapy of urinary tract infections with cephaloglycin must be mainly attributed to the antibacterial activity of this metabolite. At the present time, it is not possible to assess what influence low amounts of unaltered cephaloglycin have on the outcome of therapy.

Cephaloglycin is a broad-spectrum, orally absorbed, cephalosporin antibiotic (16). As with cephalothin (9), the cephaloglycin molecule possesses an acetyl group that can be removed by nonenzymatic hydrolysis or by esterase enzymatic activity. Cephaloglycin is also partially deacetylated in humans (5, 13). Desacetylcephaloglycin, a biologically active metabolite, can be prepared by deacetylation of cephaloglycin with a citrus acetyl esterase. The yields of crystalline compound using this complex procedure are very low. Nevertheless, limited quantities of desacetylcephaloglycin were prepared to assess the extent of deacetylation after oral administration of cephaloglycin to humans. In addition, data pertaining to chemical and biological comparisons of cephaloglycin and its metabolite are presented.

MATERIALS AND METHODS

Antibiotics. Cephaloglycin dihydrate was employed in these experiments. The desacetylcephaloglycin used was prepared in the Lilly Laboratories by S. Kukolja (8).

Microbiological assays. Disc-plate assays with Bacillus subtilis strain ATCC 6633 or Sarcina lutea strain PCI-1001-FDA were used to determine concentrations of cephaloglycin or desacetylcephaloglycin in human serum or urine. Serum specimens were drawn at 0, 1, and 2 hr after oral administration of single doses of 500 mg of cephaloglycin to male volunteers. Urine was collected for periods of 0 to 2, 2 to 4, and 4 to 8 hr. All specimens were assayed immediately.

Chromatography. Cephaloglycin levels in the presence of desacetylcephaloglycin in body fluids were determined by a modification of the paper chromatographic method of Hoehn and Pugh (6). Serum and urine specimens were those described above. All specimens were processed as quickly as possible. To avoid an increase of pH of serum due to loss of CO2, all serum samples and standards were maintained in an O2-CO2 (95:5% atmosphere, until they were applied to chromatographic papers. Urine specimens were kept at 4 C until used. Fifty- or 100-μliter samples of test serum were applied to Whatman no. 1 sheets with lanes of standard human serum on each side of the test lane. In all cases, the two standard lanes were applied at the same volume as the test lane and contained either 0.1 or 0.05 μg/ml. Therefore, depending on which standard solution was used or the volume applied, a standard lane received either 0.01, 0.005, or 0.0025 μg of cephaloglycin. After application of the serum to the paper, the spots were quickly dried in an air stream. The sheets were then immediately developed in one of two solvent systems; n-butanol-acetic acid-water (3:1:1) or ethyl acetate-acetic acid-water (3:1:1). Developed sheets were treated for bioautography or S. lutea plates as described by Hoehn and Pugh (6). The concentrations in the test sera were estimated by comparing the sizes of the zones of inhibition with the zones for the standards on the same bioautographic plate.

Urine specimens were chromatographed in a similar manner except that the volumes applied were less than serum and were adjusted in each case to compensate for the high and variable total antibiotic activity present in different collection periods.

Stability studies. The stability of cephaloglycin or desacetylcephaloglycin was studied by incubating solutions of the antibiotics at 4, 25, or 37 C. Samples were drawn at intervals and assayed immediately by the S. lutea disc-plate assay.

Disc susceptibility testing. Strains of bacteria were tested by three agar-diffusion susceptibility test methods in which single 30-μg antibiotic discs were used. The disc tests were ICS, Trypticase Soy Agar, and Bauer-Kirby. Specific directions are available for
these procedures (1, 4), and all three have been previously summarized in detail (14).

**Minimal inhibitory concentration determinations.** Broth-dilution and agar-dilution procedures were used to determine cephaloglycin and desacetylcephaloglycin minimal inhibitory concentrations (MIC) for all strains of bacteria. Nutrient Broth (Difco Laboratories, Inc., Detroit, Mich.) with 0.85% NaCl was used for broth-dilution tests because of better stability of cephaloglycin in this medium (11). Each tube (5-ml volume) was inoculated with one drop from a standardized suspension of the bacterial culture, resulting in final inocula of 10⁸ organisms per ml.

The agar-dilution method employed was the procedure used during The International Collaborative Study (ICS) on standardization of antibiotic sensitivity testing sponsored by The World Health Organization in 1963 to 1964 (4). Mueller-Hinton Agar (BBL) was the medium employed, and inocula were approximately 5 × 10⁸ bacteria per 0.05-ml drop applied to the agar surface.

The MIC with both test methods was determined after overnight incubation at 37 C.

**Correlation of disc test results with MIC values.** Zone diameters obtained with 30-μg discs were plotted against MIC values. A zone diameter was a measure of MIC, and the two were inversely related. The experimentally determined points fell along a regression line, which was calculated by the method of least squares.

**Experimental infections.** Strains of both gram-positive and gram-negative bacteria were used for experimental infections. Groups of eight white mice (11 to 13 g) were treated orally at 1 and 5 hr after intraperitoneal bacterial challenge. The mice were observed for 7 days; median effective dose (ED₅₀) values were calculated by the method of Reed and Muench (10).

**RESULTS AND DISCUSSION**

Preliminary chromatographic studies after oral administration of cephaloglycin to mice indicated that the administered cephaloglycin is excreted in mouse urine at a 50:50 ratio of the parent antibiotic and its metabolite (Table 1). Parenterally administered cephalothin is excreted by the mouse as 40:60 ratio of desacetylcephalothin and cephalothin (15). This ratio agreed with that reported for cephalothin in humans (9). Thus, there was nothing in the available data that would indicate a greater deacetylation problem with orally administered cephaloglycin than with cephalothin given parenterally.

The first indication of deacetylation of cephaloglycin in humans was the observation that assay values for the antibiotic in urine varied considerably with the assay organism employed. In one procedure the antibiotic content of a urine specimen assayed as 1,150 μg of cephaloglycin per ml. A 6-mm paper disc saturated with 0.02 ml of that urine specimen should have had a cephaloglycin content of 23 μg. However, zone diameters for the bacteria examined with these urine-saturated 23-μg discs were not between those zone diameters for 30- and 15-μg cephaloglycin discs as was expected but were smaller (Table 2). These results indicated that the antibiotic activity in urine is a combination of a small amount of cephaloglycin with a greater amount of another antibiotic, desacetylcephaloglycin. The latter compound seemed to exhibit an activity approximately equal to cephaloglycin against the assay organism S. lutea strain PCI-1001-FDA. However, the metabolite is less active against the bacterial cultures used in the disc test (Table 2), which explains the smaller zone diameters for these organisms.

Various assay results were obtained for human serum and urine specimens, depending on the organism used for assay (Table 3). The data led to a false assumption that there was approximately 24 to 29% of cephaloglycin in human urine and serum. This rationale was based on the differences

| Antibiotic administered | Antibiotics in urine | Approx per cent found in mouse urine |
|-------------------------|---------------------|-------------------------------------|
| Cephalothin (parenterally) | Cephalothin | 60 |
|                        | Desacetylcephalothin | 40 |
| Cephaloglycin (orally)  | Cephaloglycin | 50 |
|                        | Desacyetylcephaloglycin | 50 |

* As determined by chromatographic techniques after administration of 20 mg of cephalothin or cephaloglycin per kg.

**Table 2. Antimicrobial activity found in human urine after oral administration of 300 mg of cephaloglycin**

| Bacteria           | Strain no. | Diameter of zone of inhibition (mm) |
|--------------------|------------|-------------------------------------|
|                    |            | Urine disc | Cephaloglycin discs (23 μg<sup>a</sup> | 30 μg | 15 μg |
| Klebsiella pneumoniae | KA 14      | 11.8       | 22.3 | 19.7 |
| Escherichia coli    | EC 14      | 10.9       | 20.8 | 18.8 |
| Salmonella typhosa  | SA 12      | 15.6       | 26.6 | 24.8 |
| Shigella flexneri   | SH 3       | 11.6       | 24.1 | 22.1 |

* Content estimated on basis of saturating a 6-mm disc (0.02 ml) with urine that assayed 1,150 μg of cephaloglycin per ml.
TABLE 3. Antibiotic serum concentrations and urinary recovery$^a,b$ after oral administration of 300 mg of cephaloglycin

| Assay organism          | Antibiotic concn (µg/ml) for 2-hr serum | Urinary excretion (0 to 6 hr) | Percent recovery |
|-------------------------|----------------------------------------|-------------------------------|-----------------|
| Sarcina lutea PCI-1001 | 2.2                                    | 141.5                         | 28              |
| Streptococcus sp. (group D) | 0.52                                  | 23.5                          | 4.7             |
| Bacillus subtilis ATCC 6633 | 0.64                                  | 32.2                          | 6.5             |

$^a$ All assays were performed with cephaloglycin standard.

$^b$ Estimated cephaloglycin content calculated for serum: value from B. subtilis assay (based on the initial hypothesis that desacetylcephaloglycin was not detected by the B. subtilis assay) of 0.64/µg from S. lutea (total activity) of 2.2 = 29%; for urine: value from B. subtilis assay (cephaloglycin only) of 32.2/µg from S. lutea assay (total activity) of 141.5 = 24%.

in values obtained with S. lutea and B. subtilis assays. The S. lutea assay would detect both cephaloglycin and desacetylcephaloglycin, whereas the B. subtilis assay would detect only cephaloglycin. Thus, the cephaloglycin content could be calculated as shown in Table 3. The total values were not in question, but the values from the B. subtilis assay as only cephaloglycin could not be reconciled with results obtained from paper chromatography.

One logical explanation for the discrepancy described above was that the B. subtilis assay was misleading. Based on the original hypothesis that the B. subtilis assay detected only cephaloglycin, the assay values shown in Table 4 with this assay should have been due only to that antibiotic. However, the presence of desacetylcephaloglycin in combination with cephaloglycin had an influence on the B. subtilis assay, even though 1 µg of this antibiotic per ml would not produce a zone of inhibition by itself. The presence (per milliliter) of 0.5 µg of cephaloglycin assayed 1.2, 0.25 µg assayed 0.72, and 0.125 µg assayed 0.51. The true value and the assay value were therefore expressed as a fraction and used for correction purposes. By using this fraction to correct, the estimated contents of cephaloglycin in human urine and serum specimens that were initially estimated to be 24 to 29% now approached 8 to 10%.

Standard curves obtained with S. lutea PCI-1001-FDA, when 6-mm paper discs saturated with solutions of the indicated antibiotic concentrations are used, are presented in Fig. 1 and 2. With this method, desacetylcephaloglycin is more active than cephaloglycin against the assay organism. A serum sample that assays 1.0 µg/ml against the cephaloglycin curve assays only 0.62 µg/ml when the zone diameters for the specimen are read from the desacetylcephaloglycin curve (Fig. 1). Likewise, 1.0 µg/ml in urine assayed as cephaloglycin can be expressed as 0.84 µg of desacetylcephaloglycin per ml (Fig. 2). With the use of the test organism S. lutea PCI-1001-FDA and cephaloglycin as the standard, results are multiplied by the conversion factors 0.62 and 0.84, respectively, for serum and urine, to approximate the antimicrobial activity present in a sample as desacetylcephaloglycin. Conversion factors for other assay organisms have not been determined.

An example of a plate representing the chromatographic studies performed on serum or urine specimens from normal human volunteers is represented in Fig. 3. On each side of the specimens, known amounts of cephaloglycin were used. In this particular example, the patient's serum contains < 0.1 µg but > 0.05 µg of cephaloglycin per ml. Thus, with the use of cephaloglycin as the reference standard in an S. lutea assay procedure, the serum specimen that assayed as 1.95 µg of antibiotic content per ml would contain approximately 2.5 to 5% of cephaloglycin.

By using results from both corrected microbiological assays and chromatographic procedures, ratios of cephaloglycin and desacetylcephaloglycin content in human serum and urine.

TABLE 4. Calculation of cephaloglycin in human serum specimens by differential microbiological assay techniques$^a$

| Assay organism          | Antibiotic concn in mixture (µg/ml)$^b$ | Desacetylcephaloglycin | Cephaloglycin | Assay value (µg/ml)$^c$ |
|-------------------------|----------------------------------------|------------------------|---------------|-------------------------|
| Sarcina lutea PCI-1001  | 1.0                                    | 0.5                    | 0.5           | 2.15                    |
| Bacillus subtilis ATCC 6633 | 1.0                                  | 0.5                    | 0.5           | 1.2                     |
| S. lutea                | 1.0                                    | 0.25                   | 0.25          | 2.15                    |
| B. subtilis             | 1.0                                    | 0.25                   | 0.25          | 0.72                    |
| S. lutea                | 1.0                                    | 0.125                  | 0.125         | 1.85                    |
| B. subtilis             | 1.0                                    | 0.125                  | 0.125         | 0.51                    |

$^a$ Calculation: ratios of cephaloglycin actual value and the assay value for B. subtilis assay were 0.5/1.2, 0.25/0.72, and 0.125/0.51. The average of these values expressed as a decimal was 0.34.

$^b$ Antibiotics were combined in human sera.

$^c$ Determined from the cephaloglycin standard curves.
from two volunteers were estimated (Table 5). Although extreme caution was used to prevent additional deacetylation in specimens prior to assay and chromatography, a precise value for the cephaloglycin content in human sera or urine cannot be stated. However, the data from both microbiological assays and chromatography indicated the cephaloglycin content is between 2 and 10%. The apparent cures obtained in patients after administration of cephaloglycin must be due mostly to the antibacterial activity of desacetylcephaloglycin. At the present time, it is not possible to assess what influence the low amount of cephaloglycin still present has on the outcome of therapy.

Stability data for cephaloglycin and its metabolite are shown in Tables 6 and 7. The stability of cephaloglycin is pH dependent, with the antibiotic being most stable in mildly acidic media. For this reason, recommendations were made that MIC values for cephaloglycin in broths having pH values above 7.0 should be determined at 12 hr (16), or Nutrient Broth (pH 6.5 to 6.8) should be used (11). The chemistry involved in the destruction of cephaloglycin in broth at 37 C requires additional evaluation; however, on a chemical basis the formation of some desacetylcephaloglycin is possible. Rates of cephaloglycin degradation as high as 14% per hr in Trypticase Soy and Brain Heart Infusion broths (pH 7.2 to 7.4) have been reported (17). In these current tests (Tables 6 and 7), degradation rates for desacetylcephaloglycin are approximately half those observed for cephaloglycin. Because desacetylcephaloglycin appeared almost equally stable in all three broths (Table 7), selection of a special broth for susceptibility testing, as required for cephaloglycin, is not necessary.

Data comparing the in vitro activity of cephaloglycin or desacetylcephaloglycin are presented (Tables 8, 9, and 10). A sufficient number of cultures for each genera is included to represent the range of activity observed. Many of the cultures shown (Tables 8 and 9) are less susceptible to the antibiotics when tested in Nutrient Broth than they are by the ICS agar-dilution method. Cephaloglycin is more stable in Nutrient Broth; however, inocula sizes are greater in the
TABLE 5. Estimate of cephaloglycin content in human serum and urine

| Patient | Time (hr) | Assay method values (µg/ml) | Per cent cephaloglycin by assay | By chromatographic methods |
|---------|-----------|----------------------------|--------------------------------|---------------------------|
|         |           | S. lutea | B. subtilis |                      | Amt µg/ml | Per cent by S. lutea assay |
| Moore   | 0         | <0.05    | <0.5       | 10.9                | <0.1 > 0.05 | 2.5-5.0 |
| Serum   | 1         | 1.95     | 0.63       | 10.3                | <0.1 ≈ 0.05 | 3-4.4  |
|         | 2         | 2.3      | 0.70       | 10.7                | <5 > 2.5   | 2.5-5.0 |
| Urine   | 2 to 4    | 105      | 33         |                     |            |       |
| Giddens | 0         | <0.05    | <0.05      | 9.9                 | <0.1 > 0.05 | 1.9-3.8 |
| Serum   | 1         | 2.6      | 0.76       | 11.6                | <0.1 ≈ 0.05 | 1.9-3.8 |
|         | 2         | 2.57     | 0.88       | 6.8                 | <5 > 2.5   | 1.0-2.0 |
| Urine   | 2 to 4    | 260      | 52         |                     |            |       |

*a A 500-mg amount of cephaloglycin administered orally.
*b Calculated using decimal 0.34 described previously (i.e., B. subtilis assay value/S. lutea assay value 0.34 = per cent cephaloglycin in specimen).

TABLE 6. Stability of cephaloglycin and desacetylcephaloglycin in three broths at 37 C

| Time (hr) | Tryptase Soy Broth | Brain Heart Infusion Broth | Nutrient Broth |
|-----------|--------------------|---------------------------|----------------|
|           | C<sup>a</sup> | D<sup>a</sup> | C | D | C | D |
| 0         | 100 | 100 | 100 | 100 | 100 | 100 |
| 4         | 92  | 92  | 82 | 100 | 99 | 81 |
| 6         | 57  | 76  | 53 | 90 | 84 | 79 |
| 6         | 43  | 76  | 53 | 90 | 84 | 79 |
| 12        | 10  | 55  | 9.5 | 45 | 40 | 57 |

*a Cephaloglycin.
*b Desacetylcephaloglycin.

TABLE 7. Stability of cephaloglycin and desacetylcephaloglycin in human serum

| Time (hr) | At 4 C | At 25 C | At 37 C |
|-----------|--------|---------|---------|
|           | C<sup>a</sup> | D<sup>a</sup> | C | D | C | D |
| 0         | 100 | 100 | 100 | 100 | 100 | 100 |
| 4         | 85  | 100 | 74  | 88  | 65 | 70 |
| 6         | 80  | 100 | 69  | 80  | 39 | 60 |
| 24        | 67  | 98  | 37  | 45  | 5  | 13<sup>b</sup> |

*a Initial concentrations of 10 µg per ml.
*b Cephaloglycin.

c Approximate half-life (hr) at 4 C: C = >24, D = >24; at 25 C: C = 16, D = 20; at 37 C: C = 5, D = 8.

Nutrient Broth method. Perhaps equal MIC values would have been attained if 10<sup>5</sup> instead of 10<sup>6</sup> bacteria were used to inoculate broth tests to compare with the lighter inocula (5,000 per spot) used in the agar-dilution method. Greater end point stability and reproducibility of MIC values by the agar-dilution method makes it the preferred dilution technique.

Desacetylcephaloglycin appears more active in vitro than the parent antibiotic against *Staphylococcus aureus* strains (Tables 8 and 10). MIC values with methicillin-resistant *S. aureus* isolates are difficult to reproduce because of the heterogeneity of these cultures (19). Both antibiotics are very active against strains of *Diplococcus pneumoniae* and most streptococci (except enterococci).

Gram-negative bacilli are more susceptible to cephaloglycin than to its active metabolite (Tables 9 and 10). Most of the cultures required four to eight times more desacetylcephaloglycin for inhibition. The greatest difference in activity of the two antibiotics is noted with *Enterobacter* sp. Of particular importance for therapy of urinary tract infections are the *Escherichia coli*, *Klebsiella* sp., and *Proteus* sp. Cephaloglycin/desacetylcephaloglycin activity ratios for isolates of these organisms are generally two or four to one. The in vitro activity of the metabolite
against these organisms is similar to cephalothin, cephaloridine, and cephalaxin.

ED$_{50}$ values for oral administration of cephaloglycin and desacetylcephaloglycin in therapy of experimental bacterial infections in mice are those that would be expected from the in vitro data (Table 11). Both antibiotics appear equally effective against infections caused by gram-positive cocci. Cephaloglycin is more active than the desacetyl compound against gram-negative bacillus infections.

Because cephaloglycin is converted principally to desacetylcephaloglycin in humans, interpretation of in vitro data must take into account the activity of the metabolite. For this reason, susceptibility tests were performed with both antibiotics. One of the procedures used was the disc-diffusion technique of Bauer et al. (1), known as the Bauer-Kirby (or Kirby-Bauer) method. With this method, organisms are usually classified as "sensitive," "intermediate," and "resistant" (12). However, for drugs excreted in high concentrations in urine and for use only in urinary tract infections, both sensitive and intermediate classes of organisms fall into a sensitive category.

To serve as guides for therapy with cephaloglycin, in vitro tests must predict probable in vivo effectiveness. The range of zone sizes for predictive purposes is a result of correlation of zone diameters obtained with 30-µg cephaloglycin discs with MIC values (Fig. 4 and 5), in the light of clinical experience with the antibiotic (example in Table 12). Data in Table 12 indicate that adequate clinical bacteriological response was attained in both acute and chronic urinary tract infections caused by organisms with cephaloglycin MIC values ≤ 16 µg/ml or desacetylcephaloglycin MIC values of ≤ 64 µg/ml (Fig. 4). Regression lines in Fig. 5 are correlations of desacetylcephaloglycin MIC values with cephaloglycin zone diameters by three disc procedures.

For purposes of interpretation, resistant or sensitive organisms may be classified into two categories as is shown in Table 13. With the usual terminology used for the Bauer-Kirby method of resistant, intermediate, or sensitive, organisms identified as sensitive should show MIC values less than the antibiotic concentrations obtained in serum with cephaloglycin administration. However, because cephaloglycin is indicated for use in the treatment of urinary tract infections only, bacterial cultures having zone diameters of 17 mm or greater should be considered sensitive.

Evidence has been presented leading to the conclusion that after oral administration of cephaloglycin, antibacterial activity is present in

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**Table 8. In vitro activity of cephaloglycin or desacetylcephaloglycin on gram-positive cocci**

| Bacteria                  | Strain   | Special identity | Zone diameter (mm) for 30-µg cephaloglycin discs | Minimal inhibitory concn (µg/ml) |
|---------------------------|----------|------------------|--------------------------------------------------|---------------------------------|
|                           |          |                  | TSA method | Bauer-Kirby method | ICS method | Nutrient Broth | ICS agar dilution |
| *Staphylococcus aureus*   | V92      | Phage 47, 53, 59, 73, 75 | 24.6  | 26.7 | 28.8 | 4 | 4 | 4 | 2 |
| (benzylpenicillin-sensitive) | V102   | Phage 83 | 23.4 | 26.4 | 27.5 | 4 | 4 | 4 | 2 |
|                           | 14777    | ATCC       | 29.0 | 34.3 | 38.0 | 2 | 2 | 1 | 0.5 |
| *S. aureus*               | V32      | Phage 6, 47, 53, 54 | 20.7 | 23.0 | 25.6 | 8 | 8 | 4 | 2 |
| (benzylpenicillin-resistant) | V57     | Phage 42D, 52, 80, 81 | 21.0 | 24.4 | 28.4 | 8 | 2 | 4 | 1 |
|                           | V79      | Phage 52A, 79 | 25.0 | 27.2 | 28.2 | 4 | 2 | 4 | 1 |
| *S. aureus* (methicillin-resistant) | 3130   | Phage 53, 77 | 10.4 | 14.4 | 18.0 | 16 | 32 | 4 | 8 |
| *Streptococcus pyogenes*  | C203     | Type 3       | 35.0 | 37.0 | 39.0 | 0.12 | 0.25 | 0.12 | 0.12 |
| (group A)                 | 12385    | Type 4       | 34.0 | 32.0 | 32.0 | 0.12 | 0.25 | 0.12 | 0.12 |
| *Streptococcus sp.*       | 9943     | —             | 26.0 | 28.3 | 29.1 | 1 | 2 | 1 | 2 |
| (Viridans)                | 9961     | —             | 36.0 | 28.0 | 27.0 | 0.25 | 1 | 0.25 | 2 |
| *Streptococcus sp.*       | 9901     | —             | 11.0 | 10.0 | <6.0 | >32 | >32 | >32 | >32 |
| (group D) (Enterococci)   | 99       | —             | 48.0 | 36.0 | 40.0 | 0.06 | 0.25 | 0.25 | 0.5 |
| *Diplococcus pneumoniae*  | 1        | Type 1       | 40.0 | 31.0 | 36.0 | 0.12 | 1.0 | 0.25 | 0.25 |
|                           | 2        | Type 2       | 33.0 | 33.0 | 34.0 | 0.25 | 1.0 | 0.12 | 0.5 |
|                           | 3        | Type 14      | 36.0 | 38.0 | 38.0 | 0.5 | 1.0 | 0.25 | 1.0 |
### Table 9. In vitro activity of cephaloglycin or desacetylcephaloglycin on gram-negative bacilli

| Bacteria                | Strain         | Special identity | Zone diameter (mm) for 30-µg cephaloglycin discs | Minimal inhibitory concn (µg/ml) |
|-------------------------|----------------|------------------|--------------------------------------------------|---------------------------------|
|                         |                |                  | TSA method                        | Bauer-Kirby method | ICS method | Nutrient Broth | ICS agar dilution |
| **Escherichia coli**    |                |                  |                                  |                    |            |                |                  |
|                         | EC36           | 0125:B15         | 17.6                              | 21.8               | 25.0       | 4               | 16               | 8                | 16               |
|                         | EC35           | 0127:B8          | 18.5                              | 24.4               | 25.2       | 4               | 16               | 4                | 8                |
|                         | EC38           | 055:B5           | 20.2                              | 25.9               | 28.9       | 2               | 8                | 1                | 4                |
|                         | EC50           | 0124:B17         | 22.1                              | 28.1               | 31.1       | 2               | 8                | 0.5              | 8                |
| **Citrobacter group**   |                |                  |                                   |                    |            |                |                  |
| (Escherichia freundii)  | C17            | —                 | <6.0                             | <6.0               | <6.0       | >128            | >128             | >128             | >128             |
|                         | C25            | —                 | 11.9                             | 17.0               | 19.5       | 64              | 128              | 128              | 16               |
| **Klebsiella sp.**      | KA3            | —                 | 20.7                             | 27.2               | 29.1       | 4               | 8                | 2                | 8                |
| (non-motile)            | KA16           | —                 | 18.3                             | 25.5               | 27.8       | 8               | 16               | 2                | 8                |
|                         | KA22           | —                 | 18.8                             | 28.0               | 29.1       | 2               | 8                | 2                | 8                |
| **Enterobacter sp.**    | KA2            | —                 | 9.8                              | 24.2               | 25.6       | 8               | 128              | 4                | 64               |
| **Proteus mirabilis**   | PR3            | —                 | 19.6                             | 28.6               | 28.8       | 4               | 16               | 4                | 8                |
| (indole-negative)       | PR6            | —                 | 21.0                             | 27.8               | 28.6       | 4               | 16               | 8                | 8                |
| **Proteus sp.**         | PR1            | —                 | <6.0                             | 14.3               | 14.4       | 128             | >128             | >128             | >128             |
| (indole-positive)       | PR2            | —                 | <6.0                             | <6.0               | <6.0       | >128            | >128             | >128             | >128             |
| **Pseudomonas aeruginosa** | PS5           | —                 | <6.0                             | <6.0               | <6.0       | >128            | >128             | >128             | >128             |
|                         | SB38           | —                 | <6.0                             | <6.0               | <6.0       | >128            | >128             | >128             | >128             |
| **Salmonella sp.**      | SA2            | Typhosa           | 19.3                             | 21.2               | 24.7       | 4               | 8                | 4                | 8                |
|                         | SA5            | Typhimurium       | 17.1                             | 22.9               | 25.0       | 4               | 8                | 4                | 8                |
|                         | SA6            | Newport           | 18.8                             | 26.1               | 25.8       | 4               | 8                | 4                | 8                |
| **Shigella sp.**        | SH3            | Flexneri 2a       | 20.0                             | 26.3               | 29.2       | 4               | 8                | 1                | 8                |
|                         | SH5            | Flexneri 3        | 21.3                             | 29.0               | 32.8       | 2               | 8                | 1                | 4                |
|                         | SH9            | Boyd 7            | 21.4                             | 28.6               | 31.6       | 2               | 8                | 1                | 4                |

* Judged as different strains by drug sensitivity spectra.

### Table 10. Comparative in vitro activity of desacetylcephaloglycin and cephaloglycin

| Bacteria                | No. of strains | Fold difference in MIC<sup>a</sup> (no. of cultures) |
|-------------------------|----------------|--------------------------------------------------------|
|                         |                | Desacetylcephaloglycin more active | Equal MIC values | Desacetylcephaloglycin less active |
|                         |                | >4x | 4x | 2x | 2x | 4x | 8x | >8x |
| **Staphylococcus aureus** | 15             | 0        | 5   | 10 | 0   | 0   | 0   | 0   |
| **Escherichia coli**    | 24             | 0        | 0   | 0  | 0   | 10  | 9   | 5   |
| **Citrobacter**         |                | 3        | 0   | 0  | 0   | 1   | 0   | 2   |
| (Escherichia freundii)  |                | 4        | 0   | 0  | 0   | 0   | 0   | 0   |
| **Pseudomonas sp.**     |                | 3        | 0   | 0  | 0   | 3   | 0   | 0   |
| **Klebsiella sp.**      |                | 3        | 0   | 0  | 0   | 0   | 0   | 0   |
| **Enterobacter sp.**    |                | 11       | 0   | 0  | 0   | 4   | 5   | 2   |
| **Proteus sp.**         |                | 4        | 0   | 0  | 0   | 3   | 1   | 0   |
| **Salmonella sp.**      |                | 4        | 0   | 0  | 0   | 0   | 3   | 1   |
| **Shigella sp.**        |                | 71       | 0   | 6  | 10  | 7   | 18  | 20  |

* Minimal inhibitory concentration. By ICS agar-dilution procedure.
**TABLE 11.** Activity of cephaloglycin or desacetylcephaloglycin in vitro and in experimental bacterial infections in mice

| Bacteria                   | Strain | Challenge LD10 | Desacetylcephaloglycin | Cephaloglycin |
|----------------------------|--------|----------------|-------------------------|---------------|
| Staphylococcus aureus      | 3055   | 2,050          | 2.0 (µg/ml)             | 2.0 (µg/ml)   |
| Streptococcus pyogenes     | C203   | 65             | 0.2 (µg/ml)             | 0.1 (µg/ml)   |
| Diplococcus pneumoniae     | I      | 1,310          | 0.78 (µg/ml)            | 0.39 (µg/ml)  |
| Escherichia coli           | EC14   | 335            | 4.0 (µg/ml)             | 0.5 (µg/ml)   |
| Salmonella typhosa         | SA12   | 7,200          | 4.0 (µg/ml)             | 1.0 (µg/ml)   |
| Shigella flexneri          | SH3    | 158            | 8.0 (µg/ml)             | 1.0 (µg/ml)   |
| Klebsiella pneumoniae      | KA14   | 10,000         | 8.0 (µg/ml)             | 1.0 (µg/ml)   |

- Minimal inhibitory concentrations (MIC) were determined by the ICS agar-dilution method with the exception of streptococcus and diplococcus which were by the broth-dilution method. The median effective dose (ED₅₀) value is the twice administered dose (1 and 5 hr post-bacterial challenge).

**TABLE 12.** Clinical bacteriological response

| Cases with cephaloglycin MIC values | 153 Acute urinary tract infections (% response) | 73 Chronic urinary tract infections (% response) |
|-----------------------------------|-----------------------------------------------|-----------------------------------------------|
| <0.1 – 2.5                       | 87                                            | 83                                            |
| 3.12 – 5.0                       | 86                                            | 86                                            |
| 6.25 – 10.0                      | 83                                            | 89                                            |
| 12.5 – 16.0                      | 87                                            | 90                                            |
| 25-32                            | 75                                            | 33                                            |
| 50-64                            | 74                                            | 37                                            |
| ≥100                              | 21                                            | 11                                            |

- From records of clinical investigator reports on cephaloglycin evaluation, Eli Lilly and Company.
- Minimal inhibitory concentration.

**TABLE 13.** Suggested interpretation of zone diameters obtained with a 30-µg disc for determining susceptibility of urinary tract pathogens to cephaloglycin

| Disc procedure | Diameter of inhibition zone (mm) |
|----------------|----------------------------------|
| Bauer-Kirby    | 16 mm or less                    |
| ICS method     | 17 mm or less                    |
| TSA method     | 11 mm or less                    |

- Interpretation takes into account the active metabolite desacetylcephaloglycin.
- See Materials and Methods.
- For urinary tract pathogens only.
- Resistant, likely not to respond to therapy.
- Sensitive, likely to respond in urine where antibiotic is present in high concentration.

**FIG. 4.** Regression lines for zone diameters obtained with 30-µg cephaloglycin discs using the Bauer-Kirby disc procedure and minimal inhibitory concentrations (MIC) by the ICS agar-dilution method. Both lines were calculated by the method of least squares. The experimental points for calculating the lines are not shown. The slope and intercept for line 1 are -0.407 and 12.626; for line 2, these values are -0.298 and 8.739.

**FIG. 5.** Regression lines for zone diameters obtained with 30-µg cephaloglycin discs using three disc-diffusion methods and desacetylcephaloglycin minimal inhibitory concentrations (MIC) by the ICS agar-dilution method. Zone diameters for line 1 were by the TSA method, for line 2 by the Bauer-Kirby procedure, and for line 3 by the ICS disc technique. All lines were calculated by the method of least squares. The experimental points for calculating the lines are not shown.
the human primarily as the active metabolite desacetylcephalothin. Successful therapy of bacterial infections in the urinary tract must be mainly due to the antibacterial activity of the metabolite.

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ADDENDUM IN PROOF

The Food and Drug Administration has recently requested that a cephalothin 30-μg disc be used for testing susceptibility to four cephalosporin antibiotics. Suggested interpretation of zone diameter obtained with a cephalothin disc are presented in Table 14.

**Table 14. Suggested interpretation of zone diameters obtained with a 30-μg cephalothin disc for determining susceptibility to cephalothin, cephalexin, desacetylcephalothin, cephaloglycin, and cephalosporin antibiotics.**

| Cephalothin disc | Quantitative disc procedure (Bauer-Kirby) (diameter of inhibition zone, mm) |
|------------------|--------------------------------------------------------------------------------|
| Resistant        | Intermediate susceptibility | Susceptible |
| Cephalothin      | 14 mm or less | 15–17 mm | 18 mm or more |
| Cephaloridine    | For urinary tract only | Infections |
| Cephalexin       | Resistant | Susceptible |
| Cephaloglycin    | 14 mm or less | 15 mm or more |

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