Pharmacokinetic Studies in Animals and Humans of a New Cephalosporin, the Sodium Salt of 7-Cyanacetamidocephalosporanic Acid

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The sodium salt of 7-cyanacetamidocephalosporanic acid (CAA) had a relatively short serum half-life in rabbits (21 min by intravenous and 30 min by intramuscular administration) and in humans (33 min by intravenous injection). The drug was not extensively bound (about 35%) to serum in either species. Even after large doses (500 mg/kg), CAA was not well absorbed from the gastrointestinal tract of rabbits. Urinary excretion of antibacterial activity was rapid after intravenous and intramuscular administration in rabbits and after intravenous administration in men. Expressed as unchanged CAA, antibacterial activity appeared in the urine to the extent of 84% for humans, 35% for rabbits and 32% for rats. Excretion proceeded partly by active renal tubular secretion in rabbits. In this latter species, low concentrations of the active drug were detected in cerebrospinal fluid and bile after an intravenous dose of 25 mg/kg. CAA was well tolerated after intravenous administration of a single dose in both rabbits and humans.

CIBA 36,278-Ba, the sodium salt of 7-cyanacetamidocephalosporanic acid (CAA), is a new water-soluble derivative of cephalosporin C. Its antibacterial activity has been described by various workers (1, 4-7; A. D. Russell, Microbios, in press); it is bactericidal in nature, and its mode of action appears to be typical of that of β-lactam antibiotics (6, 7; Russell, in press). It is less active than cephaparin or benzylpenicillin against penicillin-susceptible strains of Staphylococcus aureus, but is more active than these antibiotics in cephalaxin, but not cephalothin, against large inocula of β-lactamase-producing strains (5, 6; Russell, in press). The activity of cephaloridin and benzylpenicillin, but not CAA, against such strains is markedly influenced by inoculum size. CAA is less active than cephaparin or ampicillin but rather more active than cephalothin against gram-negative β-lactamase-producing strains. However, all of these antibiotics are ineffective against R+ or carbenicillin-susceptible strains of Pseudomonas aeruginosa.

This report describes pharmacokinetic studies of CAA in animals and humans. Brief details have been reported previously (2).

MATERIALS AND METHODS

Drugs. CAA, 500 mg in vials, was kindly supplied by CIBA-Geigy Ltd., Basel, Switzerland. Probenecid, as the pure free acid, was a gift from Merck, Sharp and Dohme Ltd., Hoddesdon, Herts., England. For injection, probenecid was converted into its water-soluble sodium salt. Pentobarbitone sodium (Nembutal) was purchased from Abbott Laboratories Ltd., Queenborough, Kent, England.

Animals. Male albino rabbits (1.2 to 2.8 kg) were supplied by Norfolk Rabbitries Ltd., Attleborough, Norfolk, England, and male albino rats were supplied by A. Tuck and Son, Essex, England.

Animal studies. For administration to animals, CAA was dissolved in pyrogen-free 0.9% (wt/vol) Sodium Chloride Injection, B.P., the concentration for parenteral administration being 100 mg/ml and for oral administration 200 mg/ml.

In a pilot experiment, CAA (25 mg/kg) was given intravenously (iv) to an unanesthetized and a pentobarbitone-anesthetized rabbit. No marked difference between the animals was found in the concentrations of CAA in serum over 4 h. As the rather large volumes of blood (5 ml) required for bioassay were more easily collected from anesthetized rabbits, all animals were so prepared in experiments in which drug concentrations in serum were determined.

Rabbits were lightly anesthetized with pentobarbi-
tone sodium (30 mg/kg, iv). Blood samples (5 ml) were taken by cardiac puncture before, and at various times after, the administration of a single dose of CAA (25 or 50 mg/kg) either iv (marginal ear vein), intramuscularly (left gluteus muscle), or orally (by stomach tube; dose, 500 mg/kg). In two animals, probenecid (30 mg/kg iv) was administered immediately prior to CAA (25 mg/kg iv) and blood was collected as described above. The serum was assayed by a microbiological method to determine the concentration of CAA. The serum half-life of CAA was calculated from the slope of the log serum concentration/time lines. The slope of each line was determined by regression analysis with the use of the mean serum levels for each group between 10 and 60 min for the intravenous route and between 15 and 60 min for the intramuscular route.

Urine was collected from rabbits anesthetized with pentobarbitone sodium (35 to 50 mg/kg, iv) via catheters in both ureters after the administration of CAA (25 mg/kg, iv). Urine was also collected from unanesthetized rabbits receiving CAA (25 mg/kg, iv) and placed individually in galvanized metal metabolism cages for 48 h. The urine collection flask was maintained at a low temperature by means of an ice bath. Male albino rats were given CAA (25 mg/kg, iv) and placed in all-glass metabolism cages for the collection of urine over 24 h. The collection flask was kept cold during this period. Urine collected from the metabolism cages was filtered prior to microbiological assay.

Cerebrospinal fluid (CSF) was collected by puncture of the cisterna magna from pentobarbitone-anesthetized rabbits (35 to 50 mg/kg, iv) receiving CAA (25 mg/kg) either iv or intramuscularly. Samples contaminated with blood were discarded, and only one sample was taken from each animal.

Bile was collected from anesthetized rabbits (pentobarbitone sodium, 35 to 50 mg/kg, iv) by means of a catheter placed in the common bile duct before and after administration of CAA (25 mg/kg, iv). The concentration of CAA in bile and CSF samples was determined by microbiological assay.

Human volunteer studies. For administration to man, CAA was dissolved in water for injections, B.P., to give a concentration of 50 mg/ml. Five healthy adult male subjects received CAA (500 mg, iv) injected over 1 to 2 min. Blood samples were taken by venipuncture, and urine was collected for up to 10 h. Serum and urine samples were assayed microbiologically. The serum half-life of CAA in the subjects was calculated as described for rabbits from drug concentrations in serum between 0.25 and 2.0 h inclusively.

Microbiological assay. The test organism was Bacillus subtilis NCTC 8236. Assays of CAA were conducted by the cup-plate method with large plates (internal dimensions, 43 by 28 cm) of nutrient agar (Oxoid). Zones of inhibition after 24 h of incubation at 37 C were measured, and the potency of the various samples was calculated by reference to standard curves of CAA obtained in the presence of the appropriate menstruum.

Serum binding. The minimal inhibitory concentration of CAA preventing growth of S. aureus NCTC 6751 (Oxford) was determined after 48 h at 37 C in broth and in pooled serum from either rabbits or human male subjects. The extent of binding of CAA to serum protein was determined as described by Rolinson (3).

Chromatography. Samples of authentic CAA in solution, urine, and protein-free serum (0.2 to 2 μl) were applied to prepared thin-layer sheets (20 by 20 cm) of silica gel (Eastman Chromagram no. 6060). The plates were developed in either (A) n-butanol-acetic acid-water (75:7.5:2 vol/vol) or (B) n-butanol-pyridine-acetic acid-water (42:24:4:40, vol/vol). After development, the plates were dried in a stream of cold air until all of the solvents had been removed. Antibacterial activity was located by a bioautographic technique with the use of agar plates seeded with B. subtilis NCTC 8236. The volumes of the various solutions applied to the chromatograms contained sufficient antibacterial activity (in the range equivalent to 1 to 4 μg of CAA) to give inhibition zones 10 to 20 mm in diameter on the bioautographs under the conditions employed. The Rf values for CAA in solvent systems A and B were 0.2 and 0.35, respectively. The chromatographic mobility of authentic CAA dissolved in either urine or protein-free serum was identical to that of pure aqueous solutions.

RESULTS

Animal studies. Before the administration of CAA, no antibacterial activity was detected in the serum of any of the rabbits used in this study. In the two lightly anesthetized rabbits receiving CAA (500 mg/kg, orally) levels of antibacterial activity in serum were very low and in the region of the lower limit of sensitivity of the assay system (below 1 μg/ml) throughout the 4 h of the experiment. After intravenous injection of CAA (25 and 50 mg/kg) into anesthetized rabbits, antibacterial activity appeared in the blood stream in concentrations considerably higher than after oral administration. However, activity declined rapidly (Table 1). A plot of log concentration of serum activity versus time yielded approximately parallel lines for the two intravenous doses of CAA, indicating that the drug is handled by the body in a similar manner at these doses. The concentrations of serum antibacterial activity were significantly increased when probenecid (30 mg/kg) was administered prior to CAA, and the rate of decline of the serum levels was slowed in its presence (Table 1). The serum half-life (t½) values calculated for the two intravenous doses, 25 and 50 mg/kg, were almost identical (22.1 and 21.1 min, respectively).

After intramuscular injection of CAA (25 mg/kg), levels of antibacterial activity in serum reach a maximum within 15 min, after which time the level decreased rapidly (Table 2). In this latter time period, the antibacterial con-
TABLE 1. Antibacterial activity in the serum of anesthetized rabbits* receiving CAA (25 or 50 mg/kg) intravenously

| Time (min) | Antibacterial activity (μg/ml of serum) in animals treated with CAA (25 mg/kg) | Probenecid, then CAA (25 mg/kg) | CAA (50 mg/kg) |
|------------|--------------------------------------------------------------------------------|---------------------------------|----------------|
|            | Mean | Range | Mean | Range | Mean | Range |
| 10         | 28.60 | 21.9–35.0 | 40.2 | 38.3–42.1 | 51.27 | 46.9–58.9 |
| 20         | 15.65 | 9.9–19.4 | 34.75 | 34.1–35.4 | 40.13 | 35.82–44.5 |
| 30         | 12.13 | 6.63–17.0 | 28.92 | 25.6–29.24 | 24.0 | 16.66–30.13 |
| 45         | 7.87 | 3.80–10.9 | 19.16 | 17.8–20.52 | 15.24 | 10.9–18.4 |
| 60         | 5.55 | 3.52–7.12 | 17.47 | 19.94–15.0 | 10.41 | 8.79–12.03 |
| 90         | 3.35 | 1.4–3.6 | 11.65 | 12.6–10.7 | 4.17 | 3.14–5.2 |
| 120        | 1.80 | 1–3.6 | 10.12 | 9.82–11.32 | 1.57 | 1.0–2.31 |

* Five rabbits were used with CAA at 25 or 50 mg/kg; two in experiments in which probenecid (30 mg/kg, iv) was administered immediately before CAA.

TABLE 2. Antibacterial activity in the serum of five anesthetized rabbits (25 mg/kg) intramuscularly

| Time (min) | Antibacterial activity (μg/ml of serum) | Mean | Range |
|------------|----------------------------------------|------|-------|
| 5          | 8.28                                   | 5.9–9.92 |
| 10         | 12.19                                  | 9.9–16.6 |
| 15         | 14.02                                  | 11.26–16.0 |
| 20         | 12.22                                  | 8.29–14.56 |
| 30         | 8.84                                   | 6.83–13.12 |
| 45         | 6.48                                   | 4.97–8.15 |
| 60         | 5.12                                   | 3.98–6.32 |
| 90         | 2.9                                    | 1–4.4 |
| 120        | 1.8                                    | 1–2.76 |

TABLE 3. Antibacterial activity in the cerebrospinal fluid (CSF) of anesthetized animals receiving CAA (25 mg/kg)

| Route of administration | Time (min) | Antibacterial activitya | Mean | Un-boundb CAA in serum (μg/ml) | Ratio, CSF: serum |
|-------------------------|------------|-------------------------|------|-------------------------------|-------------------|
| Intravenous             | 10         | 1.52, 12.50             | 7.01 | 18.59                         | 0.38              |
| Intravenous             | 30         | 7.43, 3.97              | 5.70 | 7.88                          | 0.72              |
| Intramuscular           | 30         | 1.40                    | 1.40 | 5.75                          | 0.24              |

* Activity is expressed as micrograms of CAA per milliliter of CSF. Each result is from a different animal.
* Calculated from mean serum levels in Tables 1 and 2, and a serum binding of 35%.

TABLE 4. Biliary excretion of antibacterial activitya by anesthetized rabbits receiving CAA (25 mg/kg) intravenously

| Time interval (min) | Animal 1# | Animal 2 | Animal 3 | Probenecid-treated animalc |
|---------------------|------------|----------|----------|---------------------------|
| 0–30                | 9.47       | 26.0     | 65.6     | 24.36                     |
| 30–60               | 4.58       | 10.8     | 30.0     | 9.33                      |
| 60–90               | 0          | 0        | 0        | 6.05                      |
| 90–120              | 0          | 0        | 0        | 6.50                      |
| Total, 0–120        | 14.05      | 36.8     | 95.6     | 46.24                     |
| Percent excretion in 120 min | 0.02 | 0.06 | 0.13 | 0.08 |

* Antibacterial activity expressed as micrograms of CAA.
# Animals 1, 2, and 3: 2.5, 2.5, and 2.8 kg, respectively.
* Probenecid (30 mg/kg, iv) given to a 2.25-kg rabbit immediately before CAA.
of maximal excretion of the activity occurred within the first 30 min. Probenecid (30 mg/kg, iv) given immediately before CAA brought about a noticeable reduction in excretion of antibacterial activity in the 3-h urine.

Urine was collected from three unanesthetized rabbits in metabolism cages for up to 48 h after administration of CAA (25 mg/kg iv). Antibacterial activity was excreted in the urine collected during the first 8 h to the extent of 33.5% of the dose (mean; 32 to 37% range). No activity was detected in urine collected between 8 and 48 h. A similar pattern of urinary excretion of antibacterial activity was observed in six rats receiving CAA (25 mg/kg iv). In 24 h, 32 ± 7% (SE) of the dose was excreted in an active form, of which 95 to 98% appeared in the first 2 h.

Chromatography of urine from the rabbits and rats receiving CAA revealed the presence of only one antibacterial spot. This possessed a mobility identical to that of the unchanged drug in systems A and B.

### Human volunteer studies

After intravenous administration of CAA (500 mg) to five normal adult subjects, antibacterial activity appeared in the blood stream. However, the levels declined rapidly (Table 6), and the mean serum t½ was 32.2 min (range, 28.4 to 39.9 min). In all of the subjects, CAA was well tolerated, no discomfort being experienced either locally or systemically after dosing. Routine hematological and urinary examination did not detect any abnormalities in these subjects when measured 12 and 24 h after drug administration. CAA was bound to human serum to the extent of 33 to 36%. Chromatography of the protein-free serum from the 0.5-h sample of subject D.L. in solvent systems A and B detected only one antibacterial area on the thin-layer plates. This spot had a mobility identical to that of unchanged CAA.

The excretion of antibacterial activity into the urine of the subjects receiving CAA (500 mg iv) continued up to 10 h after administration (Table 7), although the phase of maximal excretion occurred within the first 2 h of drug administration. The mean total recovery in 10 h was 84.3% (range, 60.4 to 100.6%). Chromatography of the 0- to 2-h urine from subjects D.S. and D.L. showed the presence of only one antibacterial spot with a mobility identical to that of unchanged CAA in solvent systems A and B.

### DISCUSSION

#### Animal studies

It is evident that orally administered CAA either is not well absorbed or

![Table 5. Urinary excretion of antibacterial activity by anesthetized rabbits receiving CAA (25 mg/kg) intravenously](attachment://table1.png)

![Table 6. Antibacterial activity in the serum of human volunteers receiving CAA (500 mg) intravenously](attachment://table2.png)

![Table 7. Urinary excretion of antibacterial activity by human volunteers receiving CAA (500 mg) intravenously](attachment://table3.png)

*Expressed as micrograms of CAA per milliliter of serum.

*Activity expressed as CAA.
is degraded during absorption. On the other hand, CAA rapidly enters the blood stream after intramuscular injection and the serum $t_\frac{1}{2}$ is about 40% longer than that following intravenous administration. However, because the preparation causes considerable, but transient, discomfort to rabbits when given intramuscularly, it seems essential to formulate CAA with a potent quick-acting local anesthetic for use in man by this route.

Antibacterial activity was rapidly excreted in the urine when CAA was administered parenterally to rabbits. On the basis of the present experiments alone, it is not possible to conclude that the antibacterial activity in serum and urine is due entirely to unchanged CAA. The metabolism studies were hindered by the lack of suitable methods for the chromatographic detection of CAA and metabolites such as the desacetyl derivative. The chromatographic systems and the bioautographic location technique employed above would probably not have distinguished between CAA and the desacetyl compound and would not detect other metabolites of low or no antibacterial activity. The rapid excretion of the drug is consistent with the relatively low order of its binding to serum. The effects of probenecid indicate that CAA is excreted partly by active secretion in the renal tubules. However, from the present results it is not possible to determine the magnitude of this contribution to the overall elimination of the drug.

Metabolism of CAA to inactive products in the animal species studied is suggested by the results that only 37 and 32% of the total dose was recovered in antibacterially active form in urine of rabbits and rats, respectively, and that in the rabbit, at least, elimination of CAA via the bile was extremely small. It is possible that the site of inactivation is the liver since, in preliminary experiments, we have found that CAA is inactivated by incubation with rabbit hepatic microsomes.

**Human studies.** After intravenous administration to human volunteers, CAA was rapidly excreted in an active form, the nature of which has not been established unequivocally as an unchanged drug. The order of binding to serum was similar to that observed in rabbits. Inactivation of CAA in humans was much less than in rabbits and rats, as suggested by the high recovery of antibacterial activity in human urine. CAA was well tolerated at a dose of 500 mg iv, and no change occurred in hematological parameters or urine constituents.

Based on the previous microbiological studies with CAA (1, 4–7; Russell, in press) and the studies described here, it seems likely that CAA, given parenterally, will prove a useful addition to the chemotherapeutic antibiotics currently available.

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