Penetration of Enoxacin into Bronchial Secretions

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Enoxacin is a potent quinolone derivative with marked activity against gram-negative bacteria and staphylococci. The oral preparation has a potential role in treatment of gram-negative-bacterial lower respiratory infections if found to give adequate bronchial (sputum) concentrations. A study was done to determine the concomitant serum and bronchial concentrations of oral enoxacin after dosing with (i) 600 mg, single dose; (ii) 400 mg, single dose; and (iii) 400 mg every 12 h, four doses. Blood and bronchial secretions were collected from 20 patients predose and 2, 5, and 9 h postdose. Bronchial secretions were obtained from tracheostomies, endotracheal tubes, or bronchoscopy. Levels of enoxacin in serum and sputum were measured by high-pressure liquid chromatography. Mean peak bronchial secretion levels were similar for the 400-mg dose schedules (2.2 and 2.4 μg/ml) but were significantly higher with the 600-mg dose (4.0 μg/ml) (P < 0.05). Significant concentrations in bronchial secretions were still achievable at 9 h postdose (1.3 to 2.3 μg of enoxacin per ml). The mean ratios of enoxacin concentrations in sputum to those in serum at various time intervals for all groups were as follows: at 2 h, 0.55 ± 0.34; at 5 h, 1.04 ± 0.72; at 9 h, 0.97 ± 0.62. Considering that most gram-negative-bacterial strains are inhibited by 1.0 μg of enoxacin per ml in vitro, this study shows that oral enoxacin in practical doses achieves a concentration in bronchial secretions that is adequate to treat most gram-negative-bacterial lower respiratory infections.

MATERIALS AND METHODS

Population. Enrolled in the study were 23 patients with excessive mucopurulent sputum production and who had tracheostomy or endotracheal tube or were undergoing bronchoscopy. Patients were alert and able to communicate verbally or in writing, and informed, written consent was obtained from all patients.

Of these patients, 21 had tracheostomy or endotracheal tubes; in 2 patients, sputum was obtained from bronchoscopy. All patients had either pneumonia or tracheobronchitis.

Patients were randomized to receive oral enoxacin (supplied by Parke-Davis Canada Inc.) as follows: (i) 600 mg, single dose; (ii) 400 mg, single dose; (iii) 400 mg every 12 h, four doses. Two groups of eight patients received 400- and 600-mg oral doses of enoxacin, respectively, and seven patients received 400 mg every 12 h for four doses. Three patients were excluded from analysis because of evidence of aspiration, esophagotracheal fistula, or dilution of sputum at bronchoscopy. Two of these patients received 600-mg single doses, and one patient received the 400-mg multiple-dose regimen. Blood and sputum samples were collected predose and 2, 5, and 9 h postdose (after the last dose). Samples in the bronchoscopy patients were collected only at 2 h postdose. Sputum samples (tracheobronchial secretion) were collected undiluted directly from the trachea or bronchus by aspiration. Samples of serum and sputum were frozen at −70°C until ready for processing and analysis. The volume of sputum was measured and then mixed with twice the volume of 0.1 M phosphate buffer (pH 6.0). This mixture was then homogenized and liquefied with a Polytron Homogenizer [Brinkmann Instruments (Canada) Ltd., Rexdale, Ontario]. Laboratory tests included complete blood count, platelet count, serum creatinine, alkaline phosphatase, serum aspartate aminotransferase, and bilirubin, before and after dosing.

Assay. Serum and sputum assays for enoxacin were done by high-pressure liquid chromatography. Standards were prepared in a fashion similar to test samples, from control sputa and pooled sera, as well as phosphate buffer.
**TABLE 1. Demographic characteristics of patient groups studied**

| Dose (mg) | Mean age (yr) | Ratio of women to men | Mean wt (kg) | Mean serum creatinine (mg) | No. with pneumonia/
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<tbody>
<tr>
<td>400 mg, 1 dose (8)</td>
<td>59.4 ± 15 (30-74)</td>
<td>2:6</td>
<td>68.4 ± 16.8 (40-90.3)</td>
<td>1.3 ± 0.6 (64.3)</td>
<td>2/6</td>
</tr>
<tr>
<td>600 mg, 1 dose (6)</td>
<td>63.7 ± 10.7 (46-79)</td>
<td>3:3</td>
<td>68.3 ± 14.7 (45-83.2)</td>
<td>1.1 ± 0.2 (66.0)</td>
<td>2/4</td>
</tr>
<tr>
<td>400 mg, 4 doses (6)</td>
<td>52.5 ± 18.2 (23-72)</td>
<td>2:4</td>
<td>60.5 ± 12.4 (45-77.5)</td>
<td>0.9 ± 0.3 (88.3)</td>
<td>2/4</td>
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</table>

*a Range is given within parentheses.

*b Calculated clearance in milliliters per minute is given within parentheses.

**Chemicals.** Analytical-grade phosphoric acid (85%), tetrabutylammonium hydroxide (40%, wt/wt), and high-pressure liquid chromatography-grade acetonitrile were used in the mobile phase. Sodium hydrogen phosphate and sodium dihydrogen phosphate were used in the dilution solution. Enoxacin standard powder was supplied by Warner-Lambert Co., Ann Arbor, Mich. Penicillin G was obtained from Ayerst Laboratories, Montreal, and cefazolin sodium and tobramycin sulfate were obtained from Eli Lilly Canada Inc., Toronto.

**Liquid chromatography.** The chromatography apparatus used consisted of a model M45 pump, a column heater, a model 710B WISP autosampler, a Lambda Max-481 variable-wavelength detector, and a data module recorder-integrator (Waters Associates, Inc., Milford, Mass.). The column (30 cm by 4 mm) was packed with Techsil 10 C18 (PM Instruments Inc., Toronto, Ontario) and was preceded by a guard column packed with Bondapak C18/Corsair (Waters Associates).

The chromatographic conditions used were based on those previously described (6) for the similar compound ciprofloxacin. Elution was carried out by using a mobile phase containing 3% acetonitrile and 97% buffer. The buffer consisted of 0.025 mol of phosphoric acid per liter adjusted to pH 3 with tetrabutylammonium hydroxide. The column was maintained at 50°C with an electric column heater. Detection was at 340 nm. The injection volume was 15 µl. Retention time was 5.0 min with a run time of 7 min.

All samples and standards were diluted 1:1 with the buffer used in the mobile phase. Each sample was chromatographed in triplicate. Samples and spiked standards were analyzed simultaneously. Concentrations were calculated from calibration curves constructed by plotting peak areas against graded concentrations of enoxacin in aqueous standards. Recovery from aqueous, serum, and sputum samples was identical. The detection limit of this assay is 0.1 µg/ml.

This method is linear to at least 10 µg/ml. The following equations were derived by utilizing six standards (0.0 to 10.4 µg/ml) in the medium indicated: aqueous phosphate buffer (pH 3), \( y = 1.00x + 0.34, \text{ SE } y/x = 0.135; \) pooled serum, \( y = 0.95x + 0.65, \text{ SE } y/x = 0.514; \) pooled sputum, \( y = 1.05x + 0.11, \text{ SE } y/x = 0.339; \) where SE \( y/x \) is the standard error of the estimate.

Three slopes are not significantly different from 1.00 at the 0.05 level, and the three intercepts are not significantly different from 0.00 at the 0.05 level.

Three antibiotics were diluted in serum and tested as possible interfering substances; penicillin G at 90 U/ml, cefazolin at 120 µg/ml, and tobramycin at 11 µg/ml gave no interfering peaks under the conditions of this assay.

Each patient served as his own control. This was accomplished by comparing the enoxacin concentration of the predose sample, which should be zero, with the enoxacin concentration of the postdose samples. In none of the predose sera, and in only three of the predose sputa, were there peaks that would interfere with the determination of enoxacin. The area of the small interfering peak was subtracted from the area of the enoxacin peak for the 2-, 5-, and 9-h samples of these three patients.

**Statistical analysis.** Comparison of the means of the concentrations of enoxacin in serum and sputum of different dosage groups was done by Student's \( t \) test of unpaired data. The coefficient of correlation for the relationship between the levels in serum and sputum was calculated by linear regression.

**RESULTS**

The demographic characteristics of the patients are shown in Table 1. Ten patients had carcinoma of the larynx, tongue, or mouth and had excisional surgery with tracheostomy. None of these patients had pneumonia, but they exhibited excessive mucopurulent sputum production from tracheobronchitis. Seven patients had pneumonia, two requiring bronchoscopy and the other five requiring tracheostomy or endotracheal tube for ventilation. Six other patients had bronchitis and required ventilation postoperatively or for respiratory failure.

Ten patients received concomitant antibiotics such as penicillin, cephalosporin, or aminoglycoside. However, the high-pressure liquid chromatography assay was specific for enoxacin with no interference from the other medications. This was validated by determining the predose levels (in serum and sputum) for each patient, and by the concomitant concentrations of enoxacin in sputum and serum for the different dosage schedules (Table 2).

The concomitant concentrations of enoxacin in sputum and serum for the different dosage schedules are shown in Table 2. Peak concentrations of enoxacin in serum were achieved at 2 h postdose. The mean peak concentrations in serum after the 400-mg dose schedules varied from 2.3 µg/ml (for single dose) to 3.8 µg/ml (after multiple dose) \((P < 0.01)\). This may represent some accumulation after multiple dos-

**TABLE 2. Mean enoxacin concentrations in sputum and serum**

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Mean concn (µg/ml ± SD) in sputum/mean concn (µg/ml ± SD) in serum at:</th>
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<tbody>
<tr>
<td>2 h</td>
<td>5 h</td>
</tr>
<tr>
<td>400 mg, 4 doses (6)</td>
<td>2.1 ± 1.2/3.8 ± 0.3</td>
</tr>
<tr>
<td>400 mg, 1 dose (8)</td>
<td>1.7 ± 1.3/2.3 ± 0.8</td>
</tr>
<tr>
<td>600 mg, 1 dose (6)</td>
<td>2.5 ± 2.9/4.0 ± 1.9</td>
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ing. The mean peak concentration in serum after the 600-mg single dose was 4.0 μg/ml, not significantly higher than after multiple doses of 400 mg of enoxacin. The mean peak concentration in sputum after 600-mg doses of enoxacin (4.0 μg/ml) was significantly higher ($P < 0.05$) than the peak concentrations in sputum after the 400-mg dose schedules (2.2 and 2.4 μg/ml). Time curves of enoxacin concentration in serum and sputum for 400-mg versus 600-mg doses are shown in Fig. 1 and 2. The mean ratios of enoxacin concentrations in sputum to those in serum at the various time intervals for all groups were as follows: at 2 h, 0.55 ± 0.34; at 5 h, 1.04 ± 0.72; at 9 h, 0.97 ± 0.62.

**DISCUSSION**

Enoxacin, like other new quinolones, has major advantages over the parent compound nalidixic acid, in greater in vitro antibacterial activity (2) and improvement in pharmacokinetics and tissue distribution (1, 7). Enoxacin does have in vitro activity against many respiratory pathogens (Wise, Quinolones Bull. June:1–2, 1985) and may be useful in severe respiratory infections, even in patients with nosocomial pneumonias and cystic fibrosis. In vitro studies should be complemented with information on distribution of the drug in respiratory tract tissues and secretions.

Although the clinical significance of measuring drug concentrations in bronchial secretions is controversial, there are some recent studies correlating outcome of treatment of respiratory infections with antibiotic bronchial concentrations. For instance, the high failure rate of gentamicin in gram-negative-bacterial pneumonia has been attributed to low concentrations in bronchial secretion (8, 10). Furthermore, increasing the concentration of aminoglycoside in bronchia by direct instillation in the bronchial tree has improved the cure rate of severe gram-negative-bacterial pneumonia (8).

The results of this study suggest that enoxacin penetrates well into bronchial mucus and that sufficient concentration is achievable with practical doses to treat most respiratory pathogens except for pneumococci. With 600 mg of enoxacin, a mean peak concentration of 4 μg/ml is achieved in bronchial secretion, and significant levels are maintained up to 9 h postdose. This concentration is sufficient to inhibit nearly 100% of *H. influenzae* (MIC for 90% of isolates [MIC$_{90}$] 0.25 μg/ml), *Klebsiella pneumoniae* and other *Enterobacteriaceae* (MIC$_{90}$ 1 μg/ml), *Legionella pneumophila* (MIC$_{90}$ 0.25 μg/ml), over 90% of *Pseudomonas aeruginosa* (MIC$_{90}$ 2 to 4 μg/ml), and *Staphylococcus aureus* (MIC$_{90}$ 2 μg/ml) (2–4, 9, 11, 12).

Davies et al. (5) found similar results by measuring enoxacin in expectorated sputum. The average concentration in sputum after 600 mg of enoxacin was 3.3 μg/ml. Since expectorated sputum was used in this study, it is possible that dilution by saliva could account for the slightly lower levels. Adequate bronchial secretion or sputum levels do not necessarily reflect lung tissue levels. However, concentrations of 8 μg of enoxacin per ml in lung tissue after a single oral dose of 600 mg have been reported (13).

In summary, the broad antibacterial spectrum and the encouraging kinetic properties of enoxacin with good concentrations in sputum and lung tissue make this a promising agent in difficult-to-treat respiratory infections such as bronchiectasis, cystic fibrosis, and gram-negative-bacterial pneumonias. The oral preparation allows for outpatient treatment, earlier discharge from the hospital, and potential cost savings. Further studies are required to evaluate and confirm its full potential.

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**LITERATURE CITED**


