Determination of Pefloxacin Concentration in Mesenteric Lymph Nodes by High-Performance Chromatography

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Ten patients who had undergone laparotomies for different abdominal pathological conditions were studied to determine the levels of pefloxacin in mesenteric lymph nodes. Each patient was given 400 mg of oral pefloxacin every 12 h for the 3 days prior to surgery. Drug levels in tissue were determined by high-pressure liquid chromatography (reverse phase); the mean ± standard deviation was 17.1 ± 11.9 μg/g, with a range of 2.12 to 36.6 μg/g. This indicates an adequate pefloxacin concentration in lymph nodes and makes the drug a good option for the treatment of conditions in which lymph nodes act as an infection-promoting and/or relapse-favoring factor.

Pefloxacin is one of the newest broad-spectrum antibacterial 4-quinolones, corresponding to 1-ethyl-6-fluoro-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydro quinoline-3-carboxylic acid. It is hydrophilic, with a pH range of 3 to 4.5 in aqueous solution (16). With a single oral dose of 400 mg of pefloxacin administered to healthy volunteers, a maximum concentration (C_max) of 4.3 ± 0.2 μg/ml (mean ± standard deviation) was attained, and the time required to reach this concentration (T_max) (mean ± standard deviation) was 1.3 ± 0.3 h.

A repeated oral dose of 400 mg twice a day (b.i.d.) produces the following pharmacokinetic parameters (means ± standard deviations): C_max 10 ± 0.7 μg/ml; C_min 5.87 ± 3.72 μg/ml; T_max 1.4 ± 0.2 h; and T_1/2, 15.5 ± 1.4 h (1, 2). A steady state is reached in approximately 2.5 days. From biotransformation and excretion studies, it has been concluded that 41.7% of a pefloxacin dose is excreted unchanged through urine, 20% as an N-dimethyl pefloxacin metabolite and 16.2% as oxi-norfloxacin. It is likewise excreted through the bile unchanged as a glucuronide drug and as an N-oxide metabolite (norfloxacin), which shows the same in vitro activity as pefloxacin (15). As do the other 4-quinolones, it concentrates intracellularly, mainly inside neutrophils and alveolar macrophages (7). These characteristics make pefloxacin attractive for pathogens such as Salmonella, Brucella, Mycobacteria, and Neisseria species (8).

There have been few reports on quinolone concentrations in lymph nodes. Gehanno et al. (6) measured pefloxacin levels in one mesenteric lymph node in a patient who had been treated with 400 mg of pefloxacin b.i.d. for 3 days. They found a 14.1-μg/g concentration in this sample. These circumstances led to the conclusion that mesenteric lymph nodes are a site at which quinolones reach a high concentration, as they accumulate inside macrophages and other leukocytes (7). For this reason, pefloxacin levels were determined in mesenteric lymph nodes after repeated dosages.

Ten patients who had undergone laparotomies for different abdominal pathological conditions were included in this study; 400 mg of oral pefloxacin was administered every 12 h to each patient for 3 days prior to surgery. Mesenteric lymph nodes were removed 12 h following the last dose and stored in dry tubes at −20°C during the first hour following the laparotomy. The patients received no other antibiotics either simultaneously or during the week prior to the study. Plasmatic samples were not included in the protocol.

Pefloxacin and the internal standard were obtained from Rhone-Poulenc as reference compounds. Pefloxacin mesylate and internal standard stock solutions were prepared in distilled water at a 1-mg/ml concentration and in NaOH (0.01 M), respectively. Pefloxacin standard solutions ranging in concentration from 0.1 to 2.0 μg/ml were prepared. Tissue calibration standards were prepared by adding 0.1, 0.2, 0.5, 1.0, 1.5, or 2.0 μg of pefloxacin per ml to each milliliter of homogenate.

Tissue (100 mg) was homogenized in 1 ml of sodium phosphate buffer (0.5 M, pH 7.0) in a homogenizer at 28°C (room temperature).

By using the technique described by Montay and Tassel (11), 100 μl of internal standard at a 1-μg/ml concentration was added to 1 ml of tissue extract. The extraction was performed with 10 ml of chloroform, and the samples were mixed for 15 min. The mixture was then centrifuged at 3,500 rpm for 10 min; the organic layer was separated and evaporated under nitrogen at 60°C. The residue was resuspended in 100 μl of 1% ammonia. Finally, 50 μl of this sample was injected into the chromatograph.

A Beckman System Gold (model 126) liquid chromatograph equipped with an automatic injector, a Spectra-Physics 4270 integrator, and a UV detector, which was set at 270 nm, was used. A nucleosil C18 column (4.6 by 150 mm) (SFCC) was employed. The mobile phase was prepared with 225 ml of acetonitrile, 2 g of sodium acetate trihydrate, 1 g of citric acid monohydrate, 1 ml of triethylenediamine, and a sufficient quantity of deionized, microfiltered water constitute a total volume of 1 liter. The flow rate was set at 2 ml/min.

The recovery of pefloxacin was determined by comparing the extract from lymph nodes spiked with pefloxacin with the equivalent concentration of standard solution in 1% ammonia.

The extraction of pefloxacin and its internal standard from lymph nodes was carried out without interferences due to the extraction procedure. Under the aforementioned chromatographic conditions, the mean retention times for pefloxacin and the internal standard were 5.6 and 9.5 min, respectively. A reproducible linear calibration ranging from 0.1 to 2 μg/ml was obtained.

The correlation coefficient, slope, and intercept for one
typical calibration curve were, respectively, 0.995, 0.74238, -0.34815. The mean recovery (± standard deviation) for extraction of different pefloxacin concentrations was 93.82% ± 6%.

Intra-assay precision (calculated from repeated analyses during 1 working day) was 6.11 ± 2 within the range of 0.5 to 2 µg/ml. Interassay precision (calculated from repeated analyses on different days) was 5.4 ± 5% within the range of 0.5 to 2 µg/ml.

The study group included seven males and three females, with an average age of 47.1 years (range, 26 to 78 years); their mean weight was 68.3 kg, with a range of 50 to 79 kg.

The surgical diagnoses were cholelithiasis, four patients; abdominal aneurysm, two patients; duodenal ulcer, two patients; and pancreas and colon carcinoma, remaining two patients. Drug concentrations in lymph nodes averaged 17.1 ± 11.9 µg/g, with a range of 2.12 to 36.6 µg/g (Table 1).

New quinolones are characterized by their capacity to penetrate tissue, because of their low molecular weight, prolonged plasma half-life, low protein-binding level, and, lastly, the high concentration attained inside macrophages and neutrophils (7, 14, 15). The latter characteristic makes them specially effective in the treatment of infections by intracellular pathogens such as Mycobacteria spp., Legionella pneumophila, Salmonella typhi, Staphylococcus aureus, and others (5, 13).

Several studies have been conducted on levels of quinolones in tissues, particularly in the liver, gall bladder, brain, skin, bones, and prostate of both humans and animals (1, 5, 9, 13, 14). As these studies differ both in dosages and procedures, no adequate comparison can be established; nevertheless, it is evident that quinolone concentrations are 2- to 10-fold higher in tissue than in plasma (1, 13, 14) and that there exists a clear individual variability in drug levels in tissue (12). Brue et al. (3) evaluated ofloxacin distribution in lymph nodes in 10 patients who had received four dosages of 200 mg b.i.d. and found drug levels in tissue ranging from 0.32 to 6.36 µg/g. A clearly evident interindividual variability was observed.

Gehanno et al. (6) studied 40 hospitalized surgical patients with cervicofacial malignant tumors. All patients were administered 400 mg of pefloxacin b.i.d. during the 3 days prior to surgery. Samples from plasma and different tissues, including the salivary gland, oropharyngeal mucosa, tonsils, lymph nodes, thyroid, fat, muscle, cartilage, bone, and skin, were obtained during the surgery for determining drug levels. They found lower pefloxacin concentrations in lipids than in plasma; in contrast, concentrations were two- to fourfold higher in salivary glands, oropharyngeal mucosa, and muscles than in plasma. The highest concentrations were found in nodes, thyroid, tonsils, and cartilage. The mean (± standard deviation) pefloxacin concentration in mesenteric lymph nodes found in the present study was 17.1 ± 11.9 µg/g (Table 2). Both studies followed a similar treatment scheme, and the concentrations observed were also comparable although only one lymph node was studied by Gehanno et al. (6). Individual variability in drug levels in tissue had been reported in another work (12).

Among the patients in this study, variations in the drug concentration could be ascribable to the fact that the dosage was not adjusted to the patient’s body weight, a situation similar to that observed in comparable studies (2, 4, 10).

In conclusion, pefloxacin concentrations in the mesenteric lymph nodes of our patients ranged between 2.12 and 36.6 µg/g of tissue. These concentrations are higher than the MIC for 50% of Salmonella typhi strains tested, which have been reported to range from 0.06 to 0.25 mg/liter (17). This 4-quinolone thus appears to be a good option for the treatment of conditions in which lymph nodes act as an infection-promoting and/or a relapse-favoring factor.

REFERENCES