Enterohepatic Circulation of NY-198, a New Difluorinated Quinolone, in Rats

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Received 15 July 1987/Accepted 28 October 1987

Enterohepatic circulation of NY-198 in rats was studied. NY-198 glucuronide in the bile was rapidly hydrolyzed to NY-198 by the intestinal contents. Hydrolyzed and parent NY-198 in the bile were reabsorbed from the intestine. The amount of NY-198 in enterohepatic circulation was 59.8% of that excreted in the bile.

NY-198, [±]-1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinescarboxylic acid hydrochloride, is a new difluorinated quinolone which has broad antibacterial activity against gram-positive and gram-negative bacteria (3). The in vitro antibacterial activity of NY-198 is almost the same as those of ofloxacin and norfloxacin, but the in vivo antibacterial activity of NY-198 is greater than those of ofloxacin and norfloxacin.

Enterohepatic circulation plays important roles in the availability of drugs. Many drugs excreted in the bile of animals circulate in the liver and intestine (2, 5, 6). A few reabsorption studies on quinolones have been reported (4), but the mechanism has not been investigated.

The purpose of this study was to investigate the enterohepatic circulation of NY-198 and to attempt to clarify the mechanism.

(This study was performed in part at the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 28 September to 1 October 1986.) Male Sprague-Dawley rats weighing from 171 to 215 g were used. 14C-labeled NY-198 (specific activity, 8.4 μCi/mg; radiochemical purity, more than 99%) was used as the test compound. NY-198 and its glucuronide were measured by radioassay, high-performance liquid chromatography (HPLC), and bioassay with Escherichia coli NIHJ as the test organism. Two HPLC conditions were used. The parameters of condition A were as follows: column, TSKgel ODS-80TM 4.6 mmφ by 15 cm (Toyobo, Osaka, Japan); carrier, 0.01 M phosphate buffer (pH 2.5)–acetonitrile (8:2 [vol/vol]) containing 0.01 M sodium 1-hexane sulfonic acid; flow rate, 1.5 ml/min; detector, radioactivity monitor. The parameters of condition B were as follows: column, Nucleosil 10C18 4 mmφ by 30 cm (Nagel); carrier, 0.05 M citric acid solution–acetonitrile–1 M ammonium acetate solution (77:22:1 [vol/vol]); flow rate, 1.5 ml/min; detector, fluorescence detector (Ex. 280 nm, Em. 455 nm). The sensitivity of each assay was as follows: radioassay, 10.0 ng/ml; HPLC with condition A (measuring NY-198), 50.0 ng/ml; HPLC with condition A (measuring NY-198 glucuronide), 50.0 ng/ml; HPLC with condition B, 50.0 ng/ml; and bioassay, 200.0 ng/ml. For intra-assay precision, the coefficients of variation of the various assays were 3.6% (HPLC with condition A measuring NY-198), 0.9% (HPLC with condition A measuring NY-198 glucuronide), and 2.2% (HPLC with condition B).

14C-labeled NY-198 was administered orally to the rat at a dose of 20 mg/kg after cannulation of the bile duct. Cumulative biliary excretion of total radioactivity within 24 h after drug administration was 27.4 ± 3.3% of the dose (mean ± standard deviation; n = 5). The amounts of parent NY-198 and its glucuronide in the bile were 21.9 ± 4.7 and 63.1 ± 5.0% of the biliary excretion, respectively (measured by HPLC with condition A). Enterohepatic circulation of NY-198 in rats was studied by the following method. A 1.0-ml sample of the bile collected 8 h after oral administration of 14C-labeled NY-198 was administered intraduodenally to another rat after cannulation of the bile duct, and urinary and biliary excretions were then examined. It was found that 39.3 and 20.4% of the dose recovered in the bile (estimated by radioassay) were excreted in the urine and bile, respectively (Table 1). The amount of NY-198 that was reabsorbed (59.8% of the estimated dose recovered in the bile) was higher than the amount of ofloxacin that was reabsorbed (29.6%) (4). Since values obtained by HPLC with condition A were similar to those obtained by bioassay, it was concluded that the antibacterial activity in the urine and bile was due to parent NY-198. The radioassay value was similar to the HPLC value for the urine but was fivefold greater than the HPLC value for the bile. Even if parent NY-198 in the bile (21.9% of the biliary excretion after drug administration) was perfectly reabsorbed, the amount of reabsorbed NY-198 would be only approximately half that of NY-198 in enterohepatic circulation (59.8% of the estimated dose recovered in the bile). Consequently, the contribution of metabolites of NY-198 in the enterohepatic circulation of NY-198 was suggested. NY-198 glucuronide is the major metabolite of NY-198 in the bile; therefore, the role of NY-198 glucuronide in the enterohepatic circulation of NY-198 was investigated. Separation and purification of NY-198 glucuronide were done by means of HPLC with the following parameters: column, Nucleosil 10C18 10 mmφ by 25 cm (Nagel); carrier,

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<th>Assay</th>
<th>Cumulative amt excreted (% of dose)*</th>
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<tr>
<td></td>
<td>Urine</td>
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<tr>
<td>Radioassay</td>
<td>39.3 ± 5.9</td>
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<tr>
<td>Bioassay</td>
<td>32.2 ± 6.1</td>
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<td>HPLC</td>
<td>31.8 ± 4.8</td>
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*Results are the means of values for five rats ± standard deviations.

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acetonitrile–1% acetic acid solution (2:8 [vol/vol]); flow rate, 9.0 ml/min; detection, UV at 280 nm. The identification of NY-198 glucuronide was done by mass spectrometry. The protonated molecular ion [M+H]+ at m/z 528 (351 [molecular weight of NY-198] plus 194 [molecular weight of glucuronic acid] − 18 [molecular weight of H2O] plus H+) was presented in the mass spectrum.

The amount of reabsorbed NY-198 glucuronide was measured in the same manner as the amount of NY-198 in enterohepatic circulation was. NY-198 glucuronide solution (1.0 ml containing an equivalent of 3 mg of NY-198 was used in this study. The total cumulative amounts of NY-198 glucuronide excreted in the urine and bile within 24 h after intraduodenal drug administration were 52.2 ± 9.6% of the dose (by radioassay) and 35.8 ± 6.6% of the dose (by HPLC with condition A), respectively. These results indicate that NY-198 glucuronide plays an important role in the enterohepatic circulation of NY-198. It was also found that a portion of NY-198 glucuronide was hydrolyzed to NY-198, since NY-198 was also excreted in the urine and bile.

The in vitro hydrolysis of NY-198 glucuronide to NY-198 by intestinal contents was studied. Small-intestinal, cecal, and colorectal contents and small-intestinal mucosa were homogenized to a 10-fold volume with 1/15 M phosphate buffer (pH 7.0). A total of 1.9 ml of 1/15 M phosphate buffer (pH 7.0) was added to 0.1 ml of the purified NY-198 glucuronide solution containing an equivalent of 16 μg of NY-198. Then, 1.0 ml of the homogenate or enzyme solution containing 500 U of β-glucuronidase (E. coli; Sigma) was added, and the mixture was incubated at 37°C. A 0.2-ml sample of the incubation mixture was extracted with 5 ml of chloroform containing 5% isoamyl alcohol and measured by HPLC (with condition B). The hydrolyzing activity of the colorectal contents and that of the cecal contents were markedly higher than those of the small-intestinal contents and small-intestinal mucosa (Fig. 1). In the colorectal and cecal contents, NY-198 glucuronide was almost completely hydrolyzed to NY-198 within 1 h. The glucuronides of many other drugs are also hydrolyzed by the intestinal flora (1, 2, 5).

Gastrointestinal absorption of 14C-labeled NY-198 in rats was studied by using an in situ loop technique. Within 2 h, the amount of NY-198 absorbed from the jejunum was 90.7 ± 2.8% of the dose, while that from the colon-rectum was 33.1 ± 4.8% of the dose. NY-198 was absorbed from the lower intestine.

In conclusion, the mechanism of enterohepatic circulation of NY-198 was clarified as follows. NY-198 glucuronide, which was mostly excreted in the bile after oral administration of NY-198, was rapidly hydrolyzed to NY-198 by the intestinal contents and then reabsorbed from the intestine, together with parent NY-198 in the bile.

More than 50% of the estimated dose of NY-198 recovered in the bile was reabsorbed enterohepatically, but only 27.4% of the dose was excreted in the bile after oral administration. It was therefore suggested that the enterohepatic circulation of NY-198 contributed little to the pharmacokinetionics of NY-198 in rats.

LITERATURE CITED