Comparative Pharmacokinetics and Tissue Distribution of Norfloxacin-Glycine Acetate in Flounder, (Paralichthys olivaceus) at Two Different Temperatures

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ABSTRACT. The pharmacokinetics of norfloxacin-glycine acetate (NFXGA), a newly formulated norfloxacin, was investigated in healthy flounders at two different seawater temperature (at 12°C and 20°C) varying concentrations (100 ppm and 10 ppm), using dipping administration. It was shown that the elimination half-life (T1/2) of norfloxacin at 20°C (10 ppm: 13.95 ± 1.18 hr, 100 ppm: 11.71 ± 1.32 hr) was significantly shorter than that at 12°C (10 ppm: 16.61 ± 1.47 hr, 100 ppm: 16.32 ± 1.19 hr) in flounders. Mean residence time (MRT) was calculated at 12°C (10 ppm: 194.87 ± 29.88 hr, 100 ppm: 1,222.37 ± 161.45 hr) and 20°C (10 ppm: 168.42 ± 25.85, 100 ppm: 606.14 ± 122.75 hr). Meanwhile, in the flounder tissue distribution of norfloxacin. It was shown that serum, muscle, kidney, and liver exhibited different elimination half-lives of norfloxacin. — KEY WORDS: flounder, norfloxacin, pharmacokinetics.


Norfloxacin-glycine acetate (NFXGA) is one of the newly formulated quinolones for treatment of fish bacterial infections against gram-negative bacteria and part of gram-positive bacteria in Korea. Norfloxacin is well distributed in the body and possess good oral bioavailability in man and mammals but norfloxacin pharmacokinetic parameters derived from mammals could not be extrapolated to fish due to many differences in anatomy and physiology. Many factors are known to influence the pharmacokinetics of drugs in fish: temperature, salinity, and pH of the water, food composition, and species, etc. [4]. Recently, the effect of temperature on pharmacokinetics has been described for sulfadimidine in carp an rainbow trout, and sulfadiazine and trimethoprim in carp [12]. But up till now, there has not been reported on pharmacokinetics of norfloxacin according to change of water temperature in flounder. Therefore the objective of this study was to determine pharmacokinetic parameters of NFXGA after dipping with 10 and 100 ppm concentration, at 12°C and 20°C, respectively.

In this study, flounder (Paralichthys olivaceus) were supplied from the Aquaculture Center in the National Fisheries University, Pusan. The flounders were clinically healthy on routine veterinary inspection and fed antibiotic-free total mixed ration using accepted procedures prior to experiments. Two hundred fifty healthy flounders weighing 50 ± 10 g were kept indoors in 90 cm-deep, 1 mm2 fibre-glass tanks. NFXGA (a new salt form of norfloxacin; active ingredient, 70-75%) was supplied from Daesung Institute, Seoul. The fish were given a single dipping application in a concentration of 10 or 100 ppm of NFXGA for 1 hr at 12°C and 20°C and transferred to norfloxacin-free receptacles. Blood, kidney, liver and muscle samples were obtained from each fish at the predetermined times (0, 1, 2, 4, 6, 8, 12, 24, 36, and 48 hr) and stored in a -70°C deep-freezer before analysis of norfloxacin. High performance liquid chromatography (HPLC) was carried out for the determination of the norfloxacin concentration in serum, according to our previously reported method [6]. In order to determine norfloxacin concentration in fish tissues, microbiological assay was performed using E. coli BE 1186 as a test organism [7]. In our previous paper, it was shown that MIC (minimal inhibition concentration) of norfloxacin was 0.001 μg/ml for E. coli BE 1186 and 0.2-0.8 μg/ml for P. anguilliseptica, Edwardsiella tarda, Aeromonas hydrophila, Vibrio sp. and Pasteurella fluorescens [8].

The pharmacokinetic analysis was calculated with an aid of PCNONLIN (Statistical Consultants Inc., ver 4.0, U.S.A.) using least-square nonlinear regression analysis. Other parameters were calculated in accordance with standard procedures described by Baggott [1]. Comparisons between pharmacokinetic values at 12°C and 20°C were performed using the paired, two tailed Student’s t-test; P values < 0.05 were considered significant.

The pharmacokinetic parameters of norfloxacin in flounder serum after a single dipping administration are presented in Table 1. The Vd, is larger for all the conditions in this study, ranging from 1.7 to 2.4 L/kg, indicating the excellent cellular penetration of norfloxacin in the fish. A larger AUC was observed at 12°C (100 ppm: 51.87 ± 3.18 μg-hr/ml) compared to at 20°C (100 ppm: 35.82 ± 3.34 μg-hr/ml) (P<0.05). Generally speaking, a larger AUC indicates higher bioavailability on the one hand and more residual problems on the other hand. With respect to the AUMC which is a nonparametric pharmacokinetic value, similar tendency was observed: the value at 12°C (100 ppm: 1,222.83 ± 161.39 μg-hr2/ml) was higher as compared to 20°C (100 ppm: 607.03 ± 122.62 μg-hr2/ml) (P<0.05). MRT was calculated longer at 12°C (100 ppm: 1,222.37 ± 161.45 hr) than at 20°C (100 ppm: 606.14 ± 122.75) (P<0.05). Given these high values of MRT, it is necessary to take into consideration of setting up long withdrawal time of norfloxacin for fish, particularly flounders, from the standpoint of public health. But this long MRT has also advantage from therapeutic point of view in that norfloxacin stays long enough to treat invading pathogenic organisms. Elimination half-life of norfloxacin at 20°C (10 ppm: 13.95 ± 1.18 hr, 100 ppm: 11.71 ± 1.32 hr) was significantly shorter than that at 12°C (10 ppm: 16.61 ± 1.47 hr, 100
Table 1. Pharmacokinetic values of norfloxacin after norfloxacin-glycine acetate dipping application in flounder Mean ± S.D.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>10 ppm</th>
<th>20°C</th>
<th>10 ppm</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vdss</td>
<td>ml/kg</td>
<td>2397.03 ± 202.90</td>
<td>2355.64 ± 171.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2012.67 ± 171.51</td>
<td>1690 ± 190.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T1/2</td>
<td>h</td>
<td>16.61 ± 1.47&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.32 ± 1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.95 ± 1.18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.71 ± 1.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cmax</td>
<td>μg/ml</td>
<td>0.33 ± 0.08</td>
<td>2.16 ± 0.05</td>
<td>0.40 ± 0.01</td>
<td>2.05 ± 0.08</td>
</tr>
<tr>
<td>CLo</td>
<td>ml/kg/h</td>
<td>12.33 ± 0.87</td>
<td>1.92 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.98 ± 0.85</td>
<td>2.79 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUC</td>
<td>μg·h/ml</td>
<td>8.10 ± 0.57</td>
<td>51.8 ± 3.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.34 ± 0.59</td>
<td>35.82 ± 3.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUMC</td>
<td>μg·h²/ml</td>
<td>200.54 ± 29.45</td>
<td>1222.83 ± 161.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>173.91 ± 25.45</td>
<td>607.03 ± 122.62&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>194.87 ± 29.88</td>
<td>1222.37 ± 161.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168.42 ± 25.85</td>
<td>606.14 ± 122.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Vdss: Steady state volume of distribution; T1/2: Elimination half-life; Cmax: Maximal serum concentration; CL0: Systemic clearance; AUC: Total area under serum concentration-time curve from t0 to t∞; AUMC: Total area under the first moment curve (concentration vs time); MRT: Mean residence time

<sup>a</sup> Significant difference (p<0.05) between 12 and 20°C in 10 ppm, <sup>b</sup> Significant difference (p<0.05) between 12 and 20°C in 100 ppm.

ppm: 16.32 ± 1.19 hr, reflecting its high elimination at 20°C being the optimum temperature for flounders. The same tendency for CL0 was observed for the higher concentration of 100 ppm: the higher the temperature, the larger the CL0. But at the lower concentration, there was almost no difference of total body clearance (CL0) between two temperatures. The T1/2 of norfloxacin in serum was much longer as compared to the reported value of 3 hr in both pigs and donkeys [5, 10]. In fish, drugs like quinolones are reportedly eliminated by passive diffusion processes at the glomerular site and the gill [9]. In contrast, norfloxacin in mammals is eliminated predominantly by active renal processes (tubular secretion) resulting in a short elimination half-life of about from 3 to 7 hr in man, pigs and dogs [2, 11–12]. Also, in this study, we assume that pharmacokinetic differences between two different temperatures are related to physiological change and metabolism of norfloxacin. The effects of temperatures on the pharmacokinetic behaviors of drugs were recently demonstrated for sulfadimidine and oxytetracycline. In carp, Sulfadimidine exhibited a shorter elimination half-life at 20°C than at 10°C, indicating the important role of acetylation as metabolic pathway in carp. In other report, it has been shown that sulfamethazine and trimethoprim had a significantly shorter elimination half-life at 24°C than 10°C. The results were in well agreement with that of our results. However many investigators did not directly determine the level of the antibacterials from tissues of the experimental fishes. In present study, we determined directly the levels of norfloxacin from various tissues in flounders. The level of norfloxacin was highest in the liver, followed by the kidney, muscle, serum, irrespective of the water temperature and norfloxacin concentrations (Table 2).

On the basis of pharmacokinetic information, we concluded that NFXGA is potential clinical utility for the treatment of bacterial fish diseases and the elimination half-life norfloxacin is significantly affected by changes of water temperature in flounder, especially at high concentration (100 ppm).

REFERENCES