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In Vitro Susceptibility of the Fish Pathogen 
Aeromonas salmonicida to Flumequine

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The activity of the fluoroquinolone flumequine was investigated against the fish pathogen Aeromonas salmonicida and was compared with that of oxolinic acid. Flumequine was more active than oxolinic acid in terms of its MIC against oxolinic acid-resistant isolates of A. salmonicida and was as active as oxolinic acid against susceptible isolates. In contrast to oxolinic acid, flumequine was bactericidal, with only 1% of the bacteria surviving 6 h of exposure to the drug at concentrations slightly above the MIC. Mutation to resistance to flumequine was found to occur at a lower frequency than that to oxolinic acid. Hence, in vitro, flumequine appears to possess some advantages over oxolinic acid against this fish pathogen.

Aeromonas salmonicida is the causative agent of furunculosis, an important disease in farmed salmonids in Scotland, Norway, and Canada. The control of furunculosis is dependent upon the use of antibiotics, commonly the 4-quinolone oxolinic acid (1, 4). However, the early success achieved with oxolinic acid is increasingly being compromised by the development of resistance to the drug (5). In a previous study (2), we reported the increased activity of newer fluoroquinolones against A. salmonicida. However, it seems unlikely that some of these compounds will become available for veterinary use in the United Kingdom in the near future. In contrast, flumequine is a fluoroquinolone already used in the aquaculture industry in Norway, although it is not used in the United Kingdom. The in vitro activity of flumequine against Scottish isolates of A. salmonicida was therefore investigated to assess the potential of flumequine as an alternative to oxolinic acid.

The MICs of oxolinic acid and flumequine were determined for 83 oxolinic acid-susceptible and -resistant isolates of A. salmonicida, predominantly from natural outbreaks of furunculosis at farm sites in Scotland. MICs were determined at 22°C by agar dilution on tryptone soy agar (TSA; Oxoid) as described previously (2).

In terms of its MIC, flumequine was slightly more active than oxolinic acid against both oxolinic acid-susceptible and -resistant isolates of A. salmonicida, although the range of flumequine MICs was much broader than those recorded for oxolinic acid against resistant isolates (Table 1).

The bactericidal activities of the drugs were investigated essentially as described by Lewin et al. (7) by using two oxolinic acid-resistant isolates, MT364 and MT472, and two oxolinic acid-susceptible isolates, MT363 and MT776 (Table 2). An overnight culture of the isolates containing approximately 106 CFU/ml was diluted 1 in 50 into fresh tryptic soy broth (TSB; Oxoid) containing various concentrations of antibacterial agents. The antibacterial concentrations followed the arithmetic progression 1.5, 3, 5, and 9. Samples were taken after 1, 3, and 6 h of incubation at 22°C and were serially diluted in TSB. Percent survival was estimated by viable count on TSA. Experiments were performed in triplicate, and mean values are plotted in Fig. 1.

**TABLE 1. Range of MICs of flumequine and oxolinic acid against 83 Scottish isolates of A. salmonicida**

<table>
<thead>
<tr>
<th>Strain type (no. of isolates)</th>
<th>Test agent</th>
<th>MIC (μg/ml) at 22°C*</th>
<th>50%</th>
<th>90%</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxolinic acid resistant (38)</td>
<td>Oxolinic acid</td>
<td>3.00</td>
<td>7.50</td>
<td>1.00-15.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flumequine</td>
<td>2.00</td>
<td>4.00</td>
<td>0.10-20.00</td>
<td></td>
</tr>
<tr>
<td>Oxolinic acid susceptible (45)</td>
<td>Oxolinic acid</td>
<td>0.030</td>
<td>0.40</td>
<td>0.01-0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flumequine</td>
<td>0.075</td>
<td>0.10</td>
<td>0.075-0.50</td>
<td></td>
</tr>
</tbody>
</table>

* 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

After 1 h of exposure, neither oxolinic acid nor flumequine was bactericidal, with approximately 100% of the bacteria surviving at concentrations of up to 100 times the MIC for the bacteria (data not shown). Furthermore, oxolinic acid was merely bacteriostatic even after 6 h of exposure, with approximately 100% of the bacteria surviving (Fig. 1). In contrast, flumequine was bactericidal, killing 90% and >99% of the bacteria after 3 and 6 h of exposure, respectively, to concentrations above five times the MIC at 22°C (Fig. 1). The bactericidal activity of flumequine may be of use in eliminating the asymptomatic carrier state of furunculosis;

**TABLE 2. MICs of oxolinic acid and flumequine for isolates used in bactericidal activity and mutation frequency studies**

<table>
<thead>
<tr>
<th>Isolate*</th>
<th>Oxolinic acid (μg/ml)</th>
<th>Flumequine (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT363*</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>MT736</td>
<td>0.015</td>
<td>0.075</td>
</tr>
<tr>
<td>MT364</td>
<td>7.50</td>
<td>2.00</td>
</tr>
<tr>
<td>MT472</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>MT490*</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>MT494*</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>MT744*</td>
<td>0.03</td>
<td>0.075</td>
</tr>
<tr>
<td>MT747*</td>
<td>0.03</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* All strains were isolated from separate outbreaks of furunculosis in Scotland.

* Strains used in the mutation frequency study.

* Corresponding author.
indeed, Scallan and Smith (8) obtained promising results with flumequine in reducing carriers in salmon and trout.

Resistance to antibacterial agents is a serious problem in the aquaculture industry (5, 11), and the effective life of an antibiotic is determined largely by the rate at which resistance to it develops during therapy. Determination of the frequency of chromosomal mutation that leads to resistance can provide an indication of the rate at which resistance to a quinolone will develop (9), since plasmid-mediated resistance to the quinolones has not yet been identified clinically (3). Mutation frequencies were determined for five oxolinic acid- and flumequine-susceptible isolates of A. salmonicida, as follows. Conical flasks containing 250 ml of drug-free TSB were inoculated with the A. salmonicida isolates and were shaken overnight at 22°C. The resulting cultures were centrifuged at 11,500 × g and resuspended in 2 ml of fresh TSB to give cell concentrations in excess of 10^9 CFU/ml. Aliquots (0.1 ml) of undiluted and 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴ dilutions of the cultures were then spread onto TSA plates containing antibiotic at 5, 10, and 20 times the MIC. The plates were incubated at 22°C and were examined daily for 7 days for the presence of resistant colonies. Resistant colonies were subcultured onto TSA containing antibiotic to verify resistance, and colonies were identified to the species level by using a latex bead agglutination kit (Aquaculture Vaccines Ltd., Saffron Walden, United Kingdom).

A. salmonicida isolates developed resistance to flumequine at significantly lower frequencies than they did to oxolinic acid (Table 3). Indeed, resistance to flumequine developed at less than 1/10th the frequency that resistance to oxolinic acid did.

In conclusion, flumequine was only marginally more active than oxolinic acid in terms of its MIC. However, flumequine was bactericidal at concentrations slightly above the MIC after 6 h of exposure, whereas oxolinic acid was merely bacteriostatic. This bactericidal activity, combined with the lower mutation frequencies and better pharmacokinetics (6, 10), suggest that flumequine has several advantages as a potential alternative to oxolinic acid for use in Scotland. This is supported by the encouraging results achieved with flumequine during furunculosis therapy in Norway.

We thank the Science and Engineering Research Council for the CASE studentship to A.C.B. and the Scottish Salmon Growers Association for financial support of the project.

REFERENCES


TABLE 3. Range of mutation frequencies for five oxolinic acid-susceptible isolates of A. salmonicida

<table>
<thead>
<tr>
<th>Test agent</th>
<th>5× MIC (MIC)</th>
<th>10× MIC (MIC)</th>
<th>20× MIC (MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxolinic acid</td>
<td>3.3 × 10⁻⁹–2.64 × 10⁻⁸</td>
<td>1.7 × 10⁻⁹–6.4 × 10⁻⁸</td>
<td>&lt;1.1 × 10⁻⁸–3.2 × 10⁻⁸</td>
</tr>
<tr>
<td>Flumequine</td>
<td>1.5 × 10⁻⁹–2.0 × 10⁻⁸</td>
<td>&lt;3.3 × 10⁻¹⁰</td>
<td>&lt;3.33 × 10⁻¹⁰</td>
</tr>
</tbody>
</table>

FIG. 1. Bactericidal activities of oxolinic acid and flumequine against A. salmonicida MT363. Percent survival after 6 h of exposure to oxolinic acid (■), 3 h of exposure to flumequine (▲), and 6 h of exposure to flumequine (●) is shown.