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Aeromonas salmonicida

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In Vitro Activities of 4-Quinolones against the Fish Pathogen 
*Aeromonas salmonicida*

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The activities of five fluorinated 4-quinolones, namely, sarafloxacin, enrofloxac, PD127391, PD117596, and CI934, against the fish pathogen *Aeromonas salmonicida* were investigated and compared with that of oxolinic acid. The results indicated that with the exception of CI934, these drugs are more active than oxolinic acid in terms of MIC. No inoculum effect was observed, but the drugs were less active at 10°C than at 22°C. The presence of 3% NaCl caused an increase in drug activity. Resistance to the drugs appeared to be fairly stable, with only a small decrease in activity after 10 successive passages of the test strains on drug-free tryptone soya agar.

*Aeromonas salmonicida* is the causative agent of furunculosis, one of the most destructive bacterial diseases of farmed salmonid fish (8). As yet, a suitable vaccine against the disease is not available, and control of furunculosis is still dependent upon the use of antibiotics. Currently, conventional therapy for furunculosis consists of oxytetracycline, potentiated sulfonamide, or, more usually, oxolinic acid (3, 5). However, oxolinic acid therapy in fish farming has been compromised by the development of resistant strains of *A. salmonicida* (1, 6). Recent work has suggested that some of the new fluorinated 4-quinolones, including enrofloxac— which is already licensed for veterinary use—may be more effective in the treatment of *A. salmonicida* infections in fish than the current regimen of oxolinic acid (4, 7, 9, 13, 14; C. S. Lewin and T. S. Hastings, J. Fish Dis., in press).

In this investigation, the in vitro activities of five fluoroquinolones—enrofloxac (Bayer, United Kingdom), sarafloxacin (A-56620) (Abbott Laboratories, North Chicago, Ill.), CI934, PD117596, and PD127391 (all from Parke-Davis, Basingstoke, United Kingdom)—were compared with that of oxolinic acid (Sigma Chemical Co., Poole, United Kingdom).

MICs of each drug were determined for 38 oxolinic acid-resistant and 45 oxolinic acid-susceptible strains of *A. salmonicida* isolated primarily from outbreaks of furunculosis at Scottish salmon farms. By using the criteria proposed by Tsoumas et al. (14), strains for which the MIC of oxolinic acid was less than 1.0 μg/ml were deemed oxolinic acid-susceptible, whereas strains for which the MIC was greater than or equal to 1.0 μg/ml were deemed oxolinic acid resistant. Stock cultures were stored in tryptone soya broth (Oxoid Ltd., London, England) plus 10% glycerol at −70°C. MICs were determined by the agar dilution technique on tryptone soya agar (Oxoid) by employing an arithmetic dilution scheme devised by Smith (11). The antibiotic plates were inoculated with 10⁶ CFU per spot by using a multipoint inoculator (Denley, Billingshurst, United Kingdom). The plates were incubated aerobically overnight at 22°C in a cooled incubator (Gallenkamp, Loughborough, United Kingdom), unless otherwise stated. The MIC was defined as the lowest concentration at which growth was completely inhibited.

On average, PD117596 and PD127391 were at least 15 times more active, in terms of MIC, than oxolinic acid against oxolinic acid-resistant strains and 10 times more active against oxolinic acid-susceptible strains. Both sarafloxacin and enrofloxac were slightly more active than oxolinic acid against both oxolinic acid-resistant and oxolinic acid-susceptible strains. For the oxolinic acid-resistant isolates of *A. salmonicida*, MICs of enrofloxac were up to 5 μg/ml, while the highest MIC of enrofloxac reported by Tsoumas et al. (14) was 0.5 μg/ml. CI934 was as active as oxolinic acid against resistant strains but less active against susceptible strains (Table 1).

The effect of inoculum size on the activities of the drugs was assessed for three oxolinic acid-susceptible and three oxolinic acid-resistant strains of *A. salmonicida*. MICs were determined by plate technique as described above with inocula of 10⁶, 10⁵, and 10⁴ CFU per spot. The maximum change in MIC with inoculum size was found to be approximately threefold (1.5 to 5 μg/ml). However, considering the 10,000-fold increase in cell concentration, a 3-fold increase in MIC seems insignificant.

The MIC of each drug for 18 oxolinic acid-susceptible strains was also determined at 10°C, since outbreaks of furunculosis frequently occur at this temperature. Results were read after 5 and 7 days of incubation at 10°C. There was a two- to threefold increase in MIC at 10°C after both 5 and 7 days of incubation, compared with the MICs at 22°C.

Ion concentrations can affect the activities of the quinolones (12). The effect of salinity on the MICs was therefore investigated with seven oxolinic acid-susceptible strains of *A. salmonicida*. MICs were determined in the presence of 3% NaCl (wt/vol), which is approximately the average salinity of seawater (2, 10). After both 5 and 7 days of incubation at 22°C, there was an overall decrease in MIC compared with the MICs for strains tested in the absence of added salt. In the cases of sarafloxacin, enrofloxac, PD127391, and oxolinic acid, there was a mean threefold decrease for each. However, PD117596 showed a mean 15-fold decrease, whereas the activity of CI934 was barely affected.

It has recently been reported that resistance of *A. salmo-
nicida to oxolinic acid is reduced after repeated passage on tryptone soya agar medium (14). After 10 successive passages of 20 oxolinic acid-resistant strains of A. salmonicida on drug-free tryptone soya agar, there was a mean threefold decrease in the MIC of oxolinic acid. However, no such decrease was observed for PD117596, PD127391, or sarafloxacin, whereas a twofold decrease in MIC was noted for enrofloxacin and C1934. Despite the threefold decrease in MIC observed for oxolinic acid, all strains are still classified as resistant by the criteria of Tsoumas et al. (14), and hence the significance of such a small change must be questioned.

In conclusion, PD117596, PD127391, sarafloxacin, and enrofloxacin are at least as active, in terms of MIC, as oxolinic acid against oxolinic acid-susceptible organisms. However, all four of these drugs, but particularly PD117596 and PD127391, were considerably more active than oxolinic acid against the oxolinic acid-resistant strains. There was no inoculum effect on the MICs of the drugs, and drug resistance tended to be fairly stable, with only small changes in MIC after 10 successive passages on drug-free tryptone soya agar. However, at 10°C, at which temperature outbreaks of furunculosis in salmon are not unusual, an increase in MIC was observed for all drugs. Nevertheless, with the exception of C1934, the MICs of all drugs remained well below 1.0 μg/ml. The apparent increase in activity of the drugs in the presence of NaCl contrasts with results obtained for other bacterial species, for which the MICs of fluoroquinolones were unaffected by Na⁺ ion concentration (12). These results, combined with evidence that fluoroquinolones may be more bactericidal than oxolinic acid (Lewin and Hastings, in press), suggest that further investigations with these agents are required, since the drugs may represent a potential advance in the treatment and control of furunculosis.

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We are also grateful to the manufacturers for supplying samples of the antibiotics used in this study.

<table>
<thead>
<tr>
<th>Strain type (no. of isolates)</th>
<th>Test agent</th>
<th>MIC at 22°C (μg/ml)</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>
</tr>
<tr>
<td></td>
<td>Sarafloxacin</td>
<td>1.50</td>
<td>4.00</td>
<td>0.20-4.00</td>
</tr>
<tr>
<td></td>
<td>Enrofloxacin</td>
<td>0.50</td>
<td>1.00</td>
<td>0.05-5.00</td>
</tr>
<tr>
<td></td>
<td>C1934</td>
<td>3.00</td>
<td>7.50</td>
<td>0.30-10.00</td>
</tr>
<tr>
<td></td>
<td>PD127391</td>
<td>0.10</td>
<td>0.15</td>
<td>0.015-0.30</td>
</tr>
<tr>
<td></td>
<td>PD117596</td>
<td>0.15</td>
<td>0.50</td>
<td>0.075-0.75</td>
</tr>
<tr>
<td>Oxolinic acid susceptible (45)</td>
<td>Oxolinic acid</td>
<td>0.03</td>
<td>0.40</td>
<td>0.01-0.75</td>
</tr>
<tr>
<td></td>
<td>Sarafloxacin</td>
<td>0.05</td>
<td>0.20</td>
<td>0.0075-1.00</td>
</tr>
<tr>
<td></td>
<td>Enrofloxacin</td>
<td>0.02</td>
<td>0.15</td>
<td>0.004-0.75</td>
</tr>
<tr>
<td></td>
<td>C1934</td>
<td>0.40</td>
<td>1.00</td>
<td>0.04-3.00</td>
</tr>
<tr>
<td></td>
<td>PD127391</td>
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<td>0.03</td>
<td>0.002-0.10</td>
</tr>
<tr>
<td></td>
<td>PD117596</td>
<td>0.015</td>
<td>0.05</td>
<td>0.003-0.50</td>
</tr>
</tbody>
</table>

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

LITERATURE CITED


TABLE 1. In vitro activities of 4-quinolones against 83 A. salmonicida strains