Therapeutic Effect of Flumequine against Pseudotuberculosis in Cultured Yellowtail

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The in vitro antibacterial activity of flumequine, a synthetic quinolone antibacterial agent, against Pasteurella piscicida, which causes pseudotuberculosis in yellowtail Seriola quinqueradiata was examined. Subsequently, the in vivo effective dose of the agent and its therapeutic effect in the field were studied.

Flumequine showed an minimum inhibitory concentration of 0.1–0.39 μg/ml against 43 strains of P. piscicida out of 44 strains tested.

In examining the in vivo effective dose, 5, 10, 15 and 20 mg/kg body weight/day of flumequine were administered for 5 days to diseased yellowtail naturally infected with pseudotuberculosis. After completion of administration, the number of dead fish in the group dosed with 20 mg/kg was significantly lower than in other groups. Further, 10 and 20 mg/kg of flumequine were administered for 5 days, in the field, to diseased yellowtail with pseudotuberculosis. It was found that the numbers of dead fish in the groups administered with flumequine were lower than that in the control group, and that mortalities in the group dosed with 20 mg/kg were particularly low.

Flumequine, a synthetic quinolone antibacterial agent, has a strong antibacterial action against gram-negative bacteria1–5) and is widely used in various countries as a therapeutic drug for the treatment of infectious diseases in human beings and domestic animals caused by Escherichia coli.4)

Some reports have also been published on the antibacterial activity of flumequine against fish pathogenic bacteria6,7) and on the therapeutic effect against furunculosis in rainbow trout.7)

Pseudotuberculosis in yellowtail Seriola quinqueradiata occurs in various culture farms in Japan every year and the damage is serious. Ampicillin and oxolinic acid have been used for the treatment of this disease in these years. However, recently, drug-resistant strains have been found8,9) and it is of much concern that these drugs are becoming less effective.

The effectiveness of flumequine against pseudotuberculosis in yellowtail and its effective dose have therefore been investigated. Also, the antibacterial activity of flumequine against Pasteurella piscicida, which causes the infection, was studied in a fish culture farm.

Materials and Methods

Test Drug

Flumequine (C₁₄H₁₂FNO₃), as a crude powder, was used for the drug-sensitivity experiment and a composition containing 5% flumequine for the experiment in the field on its effectiveness against pseudotuberculosis in yellowtail.

Sensitivity Test

Test microorganisms: 44 strains of Pasteurella piscicida isolated from diseased yellowtail, with pseudotuberculosis, in culture farms in Wakayama, Kochi, Yamaguchi, Saga, Nagasaki and Kagoshima Prefectures, during 1980–1984, were used.

Measurement of minimum inhibitory concentration (MIC): Measurement of an MIC was conducted according to the MIC measurement method established by Japan Society of Chemotherapy.10) Two percent NaCl-added bouillon for sensitivity measurement (Nissui Pharmaceutical Co., LTD) was used as a medium for cell propagation and 2% NaCl-added Sensitivity Disc Medium-N (Nissui Pharmaceutical Co., LTD.) as a medium for measurement of sensitivity. The incubation temperature was 25°C and the culture period was 24 h.
**Determination of Effective Dose**

Experiments were carried out during September 10 through October 5, 1984, using groups of diseased yellowtail naturally infected with pseudotuberculosis, in a culture farm located in Yamanouchi Prefecture. Particulars of the test and control groups are shown in Table 1. Five groups of yellowtail, each consisting of about 5,200 fish (average body weight: 240 g), were held separately in 5 cages (10 m × 10 m × 7 m). After pseudotuberculosis in the yellowtail was ascertained, 5, 10, 15 and 20 mg/kg of flumequine were administered as a mixture with minced sand lance to four of the groups for 5 days. To the remaining one group was administered minced sand lance without containing the drug, which was used as a control group. The daily feeding rate was the same for each of the groups, which was 10% during the drug-administration period and 15% during the non-administration period. The water temperature during the test period was 24.3–26.9°C.

**Field Therapeutic Test**

Experiments were conducted during October 12 through 28, 1984 in a yellowtail culture farm located in Ehime Prefecture using diseased yellowtail naturally infected with pseudotuberculosis. Three cages (10 m × 10 m × 10 m) respectively containing about 6,000 yellowtail having an average body weight of 500 g were used. Group No. 1 was a control group, to which the drug was not administered at all and to groups No. 2 and No. 3 were administered 10 and 20 mg/kg of flumequine for 5 days respectively by the above described method.

**Determination of Effect**

The effect of flumequine in both the effective dose determination experiment and field therapeutic experiment was determined by the following procedure. The number of dead fish in each group during the experiment was counted and a test of significance ($\chi^2$ test) was applied based on the number of dead fish during 12 or 16 days after the completion of administration. Five to 10 fish in a dying state or just after death were sampled for necropsy from each of the cages on the days when the tests were started, when the administration was completed, and when the tests were completed. *P. piscicida* was examined in the blood of the heart and in the spleen and kidney by using 2% NaCl-added Brain Heart Infusion Agar (Nissui Pharmaceutical Co., LTD.).

**Table 1. Administered dosage of flumequine against pseudotuberculosis in cultured yellowtail**

<table>
<thead>
<tr>
<th>Administered Dosage (mg/kg B.W./day)</th>
<th>Term (days)</th>
<th>Number of fish</th>
<th>Mean of fish body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Cont.)</td>
<td>—</td>
<td>5,074</td>
<td>220</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5,295</td>
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<td>5,303</td>
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<td>5,121</td>
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</tr>
<tr>
<td>20</td>
<td>5</td>
<td>5,253</td>
<td>280</td>
</tr>
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</table>


**Results**

**Drug Sensitivity Test**

MIC values of flumequine against 44 strains of *P. piscicida* are shown in Fig. 1. Of these, 43 strains (97.7%) showed MICs of 0.1–0.39 μg/ml and only one strain (2.3%) showed an MIC of 1.56 μg/ml.

**Determination of Effective Dose**

The changes in the numbers of dead fish in the test and control groups on each day during the test period are shown in Fig. 2. A large number of dead fish were observed daily in the control group, whereas in any of the flumequine-ad-
ministered groups, the number of dead fish started decreasing 3 of 4 days after the beginning of administration and no increase of dead fish was shown after the completion of administration. Cumulative mortalities of the control group and the groups dosed with 5, 10, 15 and 20 mg/kg during 16 days after the completion of administration were 6.12, 1.28, 0.56, 0.24 and 0.04%, respectively. Thus, there was a tendency that with higher dosages of flumequine, the cumulative mortality was lower. Table 2 shows the result of test of significance with respect to the number of dead fish in each group after the completion of administration and Fig. 3 illustrates daily mortality of each group after the

Fig. 2. Number of dead fish by flumequine dosage.

(↓: administration).

Fig. 3. Daily mortality after completion of medication by flumequine dosage.
completion of administration. A significant difference ($P<0.001$) was observed between the numbers of dead fish of each of the test groups on the one hand and the control group on the other. Further, among the flumequine administered groups, there was a significant difference between the group dosed with 5 mg/kg and those dosed with 10 mg/kg or more ($P<0.001-0.05$) and between the group dosed with 10 or 15 mg/kg and that dosed with 20 mg/kg ($P<0.01$). Thus, the dose response relationship was established.

Table 2. Test of significance of number of dead fish after completion of drug administration

<table>
<thead>
<tr>
<th>Dosage (mg/kg B.W./day)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
<td>$P&lt;0.001$</td>
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<tr>
<td>0</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
<td>20</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 3. *P. piscicida* detection in each of the groups during the test period

<table>
<thead>
<tr>
<th>Dosage (mg/kg B.W./day)</th>
<th>Before the administration</th>
<th>The completion of administration</th>
<th>16th day after the completion of ad.</th>
<th>30th day after the completion of ad.</th>
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<tbody>
<tr>
<td>0</td>
<td>8/10*</td>
<td>7/10</td>
<td>3/5</td>
<td>5/10</td>
</tr>
<tr>
<td>5</td>
<td>6/10</td>
<td>3/9</td>
<td>1/5</td>
<td>2/10</td>
</tr>
<tr>
<td>10</td>
<td>8/10</td>
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</tr>
<tr>
<td>20</td>
<td>9/10</td>
<td>0/10</td>
<td>0/5</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* Number of fish, positive/examined.

Field Therapeutic Test

Fig. 4 shows the state of daily changes in the number of dead fish in the test and control groups during the test period. A large number of dead fish was observed in the control group throughout the test period. On the other hand, in the flumequine-administered groups, the number of dead fish decreased from the 4th day after the start of administration with respect to 5, 10 and 15 mg/kg dosed groups and from the 2nd day with respect to the 20 mg/kg dosed group, and the number of dead fish did not increase after the completion of administration.

The number of dead fish in each group after the completion of administration was statistically analysed. As the result, a significant difference was revealed between the control group and 10 mg/kg dosed group ($P<0.005$) and 20 mg/kg dosed group ($P<0.001$) and between 10 mg/kg dosed group and 20 mg/kg dosed group ($P<0.05$).

During the test period, *P. piscicida* was detected in the control group at a high rate throughout the test period whereas the bacteria were not detected in the test groups after the completion of administration.
Discussion

Ampicillin has long been used for the treatment of pseudotuberculosis in yellowtail because the drug has had high activity against *P. piscicida* which is the pathogen of the disease. However, in recent years there have been found strains which show resistance to ampicillin and, a desired therapeutic effect has not been obtained.

The authors have made studies on the MIC of flumequine against *P. piscicida*. The substance showed an MIC of 0.1–0.39 μg/ml against 98% of the strains tested. Antibacterial activity of flumequine against *P. piscicida* in the present study is almost equal to or higher than that in the previous report. It was therefore decided to confirm the effect of flumequine in vivo on pseudotuberculosis in yellowtail.

The authors made studies on an effective dose of flumequine against pseudotuberculosis in yellowtail and found that the number of dead fish in each test group was significantly lower compared to the control group and that *P. piscicida* was not detected at all after the completion of administration in the groups dosed with 10 mg/kg or more. From these results, flumequine is considered to be effective against pseudotuberculosis. Further when comparison was made between the numbers of dead fish in the different dose groups after the completion of administration, the number in the 5 mg/kg-dosed group was significantly larger than those in the groups dosed with 10–20 mg/kg and also a significant difference was found between the groups dosed with 10 and 15 mg/kg and that dosed with 20 mg/kg. Thus, a dose response was observed. In any of the present experiments, a dose of more than 20 mg/kg was not employed. However, since almost no dead fish were found after the completion of administration in the 20 mg/kg-dosed group and since *P. piscicida* was not detected at all from this group, it is considered that a daily dosage of 20 mg/kg is appropriate for the therapeutic treatment of pseudotuberculosis in yellowtail.

Michel et al. reported that as the result of administration of 6, 12, 24 mg/kg body weight/day of flumequine for 6 days for the control of artificial challenge with *Aeromonas salmonicida* in rainbow trout *Salmo gairdneri* and searot *Salmo trutta*, therapeutic effect was observed in all the administered groups and suggested a recommended dose of 12 mg/kg against this infection. The dose of flumequine against furunculosis in salmonid recommended by Michel et al. was somewhat less than against pseudotuberculosis in yellowtail the present authors suggest. This is considered to be due to the differences in the species of fish and pathogens and in the process of infection, i.e. artificial challenge and spontaneous infection. Further, in the field experiment conducted by the present authors, the significant difference in the numbers of dead fish was observed between the group administered with 10 mg/kg and that administered with 20 mg/kg. This may support the appropriateness of the recommended dose of 20 mg/kg.

In order for flumequine to be practically used in future, it may be necessary to make the further investigation on the distribution and the residual property of this drug in the organs of yellowtail.

References

9) Nanseiikaiku Block Kaigi: Report on Fish Culture Research in the Group of South-Western Sea Area No. 5, 64–68 (1982).