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OXolinic Acid in the Trout: Bioavailability and Tissue Residues

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Résumé

L’ACIDE OXOLINIQUE CHEZ LA TRUITE : BIODISPONIBILITÉ ET RÉSIDUS TISSULAIRES. — Une étude de la biodisponibilité sérique et de l’élimination tissulaire de l’acide oxolinique est effectuée chez la truite. La molécule est administrée, incorporée dans l’aliment, à la posologie de 12 mg/kg/j durant sept jours consécutifs. L’étude réalisée a nécessité la mise au point d’une technique analytique par chromatographie liquide haute performance, dont le descriptif est détaillé. Les résultats obtenus démontrent le maintien de concentrations sériques supérieures aux CMI des germes pathogènes cibles (Aeromonas et Yersinia) durant toute la durée du traitement. Il en est de même au niveau tissulaire. Par ailleurs, en ce qui concerne le problème des résidus d’acide oxolinique dans les tissus consommables (masse musculaire et peau) sur la base d’un seuil de tolérance de 0,05 ppm, un temps d’attente de six jours après l’arrêt du traitement tel qu’il est préconisé peut être envisagé.

At the moment there is a recrudescant interest for the application of quinolones in veterinary medicine. Most certainly, this development can be attributed to their antibacterial activity, which is primarily centred on Gram negative bacteria, and a compatible price.

In the field of fish farming, two compounds are especially used, oxolinic acid and flumequine. Studies were published on the latter by Michel et al (1980), Chevalier et al (1981), and Keck (1987). The use of oxolinic acid in fish farming is supported by only a very limited number of publications. Endo et al (1973a) report that oxolinic acid exerts remarkable antibacterial activity against the major pathogens in fish. The mean values obtained are as follows: Aeromonas salmonicida, 0.02 μg/ml; Aeromonas hydrophila, 0.10 μg/ml; Vibrio anguillarum, 0.05 μg/ml; Chondrococcus columnaris, 0.40 μg/ml.

Oral doses of 10 to 30 mg/kg/day for 5 consecutive days are claimed to be therapeutically effective in carp, which has previously been experimentally infected with Aeromonas hydrophila. The authors also indicate that the LD50 in this species is > 4 000 mg/kg.

In their further study, the authors report that, in trout, Aeromonas salmonicida can be controlled by oxolinic acid administered at a dose-level of 5 mg/kg/day for 5 consecutive days (Endo et al 1973b).

The usefulness of the quinolones and especially oxolinic acid, for the control of Vibrio infections of marine fish has also been attested (Austin et al 1981, 1982). These studies also include appetite tests performed in the turbot.

The compound is of paramount importance for the control of two bacterial diseases which are often found in salmonids, ie Yersinia ruckeri and Aeromonas salmonicida.

Rodgers and Austin (1983) determined a MIC of the oxolinic acid ranging from 0.1 to 1 μg/ml against Yersinia ruckeri.

The treatment, implying the drug supplementation of the trout diet with a dose of 10 and 20 mg/kg/day for 10 consecutive days, eliminates the bacterial infection. According to Austin et al (1983), the furunculosis pathogen of the trout, Aeromonas salmonicida, has a MIC < 1 μg/ml. The experimental infection of the fish showed the evidence of the curative action of the compound for a dosage-level ≥ 5 mg/kg per day for 10 consecutive days.

The tolerance of the fish, ie Seriola quinquergata, to the antibiotic has been fully described by Miyazaki et al (1984). Severe toxic manifestations appear only after administrations of doses of 120 mg/kg/day.

Quite recently, De Grandis and Stevenson (1985), mentioned the activity of oxolinic acid against Yersinia ruckeri expressed in the form of a MIC0 ≤ 0.5 μg/ml.

We conducted a study confirming the activity of the compound against fresh fish culture isolates of Yersinia ruckeri.
Endo et al. (1973a) carried out pharmacokinetic studies on the blood and tissue distribution of oxolinic acid in carp. The various singly administered oral doses resulted in the appearance of a serum level plateau which could be sustained for several hours to several days according to the dosage-level. Elimination was slow. There was no accumulation of the compound in muscle and kidney contrary to what was observed in liver and pancreas.

Keck (1987) compared the activity of oxolinic acid and flumequine against the principal bacterial pathogens in fish and gave some indications on the serum and tissue pharmacokinetics of oxolinic acid in trout. An experimental infection with Yersinia ruckeri confirmed the interest of the use of quinolones for the treatment of bacterial infections in fish.

We studied the serum bioavailability and tissue residue elimination of oxolinic acid after administration of the medicated diet to trout under standard fish farm conditions.

**Materials and Methods**

1. **Animals**

Rainbow trout (about 350), weighing approximately 200 g were used in this experiment. The fishes were kept under usual fish farm conditions, in water at a temperature of 9-10 °C originating from a source supplying the complete fish tank installations. The latter were located in Roquebillère 06 (Centre de Pisciculture de la Fédération Départementale des Associations de Pêche et de Pisciculture des Alpes-Maritimes).

2. **Product**

The oxolinic acid was added to the trout feed by using a preparation containing 24 % of the antibacterial agent (Oxomid 24®, Virbac 06516 Carros).

3. **Experimental procedure**

All the fish were given the feed containing the oxolinic acid. They were fed once a day in the morning at the feeding rate of 1 % body weight. The dose-level applied corresponded to 12 mg/kg/day for 7 consecutive days.

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**Table 1 – Serum concentrations in the trout during treatment (12 mg/kg/day)**

<table>
<thead>
<tr>
<th>Time of sampling (hours)</th>
<th>Concentration (µg/ml) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before administration</strong></td>
<td>00</td>
</tr>
<tr>
<td><strong>After administration</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>8</td>
<td>0.23 ± 0.16</td>
</tr>
<tr>
<td>24</td>
<td>0.46 ± 0.29</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.73 ± 0.66</td>
</tr>
<tr>
<td>8</td>
<td>0.61 ± 0.18</td>
</tr>
<tr>
<td>24</td>
<td>0.38 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.54 ± 0.10</td>
</tr>
<tr>
<td>8</td>
<td>0.62 ± 0.14</td>
</tr>
<tr>
<td>24</td>
<td>0.50 ± 0.13</td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.51 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>0.65 ± 0.16</td>
</tr>
<tr>
<td>4</td>
<td>0.60 ± 0.30</td>
</tr>
<tr>
<td>6</td>
<td>0.68 ± 0.42</td>
</tr>
<tr>
<td>8</td>
<td>0.63 ± 0.21</td>
</tr>
<tr>
<td>10</td>
<td>0.48 ± 0.18</td>
</tr>
<tr>
<td>16</td>
<td>0.46 ± 0.23</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.57 ± 0.31</td>
</tr>
<tr>
<td>10</td>
<td>0.50 ± 0.22</td>
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<tr>
<td>24</td>
<td>0.52 ± 0.22</td>
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<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.63 ± 0.25</td>
</tr>
<tr>
<td>10</td>
<td>0.74 ± 0.58</td>
</tr>
<tr>
<td>24</td>
<td>0.55 ± 0.13</td>
</tr>
</tbody>
</table>
4. Samples

Blood samples were performed on dry tubes after decapitation of the fish, at a rate of 10 fishes at each time. The serum obtained after centrifugation was immediately frozen at \(-20^\circ\text{C}\) and stored pending analysis.

The chronology of blood samples is as follows: prior to the first administration; 3, 8, 24 h after the first, the second and the third administration; 1, 2, 4, 6, 8, 10 and 16 h after the fourth administration; 5, 10 and 24 h after the fifth administration; 5 and 10 h after the sixth administration; 24 h after the seventh and last administration.

The tissue samples were collected only from the skin and muscle mass, the only edible parts of the fish. After they had been eviscerated and rinsed, the trouts (n: 10 at each time) were frozen at \(-20^\circ\text{C}\) and stored till analysis.

The chronology of the tissue samples is as follows: prior to the administration; 8 h after the first administration, 3 h after the second administration, 8 h after the third administration, 6 and 16 h after the fourth administration, 5 h after the sixth administration; 24, 48, 96, 144 and 240 h after the seventh and last administration.

5. Analysis of oxolinic acid

It was performed after a previous extraction by high performance liquid chromatography (HPLC).

*Serum*: 100 µl of serum and 6 ml of chloroform were poured into a 10 ml centrifugation tube, stirred for 1 min with a Vortex type agitator and then centrifuged for 5 min at 3500 t/min. The aqueous phase was removed. The organic phase was transferred in a 25 ml round-bottom flask and evaporated under vacuum. The dry extract was rinsed with 1 ml of mobile phase. 100 µl was injected in the loop.

*Tissues*: 5 g of thinly cut tissues (muscle and skin) were placed with 0.25 ml of distilled water (allows the supplementation of the tissues for the determination of the standard curve) in a Virtis bowl and let to stand for 30 min. 0.5 ml of an aqueous solution of 1N hydrochloric acid was added and let to stand for 5 min. 50 ml of ethyl acetate were added, homogenized with a Virtis 45 for 5 min, filtrated on a 4 porosity in a 100 ml round-bottom flask and then evaporated to dryness under vacuum. The dry extract was rinsed with 1 ml of mobile phase. 100 µl was injected in the loop.

The compound administered in the feed under standard breeding conditions at a dosage-level of 12 mg/kg/day, enabled to maintain serum and tissue levels which were compatible with the MIC of oxolinic acid against these two bacteria was lower or equal to 0.10 µg/ml.

The analytical technique used, allows the detection of concentrations < 0.010 µg/ml or g with a yield of 92 % for the serum and 80 % for the tissues.

Results

Of 10 different strains tested, four of them were found resistant (MIC ≥ 3.1 µg/ml) but most of them showed a mean MIC of 0.10 µg/ml.

The serum concentrations of oxolinic acid obtained in the trout during the treatment period are shown in table 1. Results show that the distribution of the compound is slow and the concentrations reach 0.46±0.29 µg/ml only 24 hours after the first administration. Then, levels approaching 0.5 µg/ml are sustained throughout the treatment period.

The tissue concentrations, in the treatment period and after the administration was discontinued, appear in table 2. Results obtained were significant and showed the distribution of the compound in the muscle mass and skin. Moreover, concentrations equivalent to maximal two-fold higher values than the corresponding serum levels were sustained at approximately 1.4 µg/ml. Then, the tissue elimination occurred rapidly for the few days following the interruption of the administration. Concentrations lower than 0.1 µg/g were analysed on and after day 4.

Discussion

For the salmonid farming industry, and especially for the trout, the major bacterial disease encountered by the fishculturers is furunculosis caused by *Aeromonas salmonicida*, and yersiniosis or redmouth diseases due to *Yersinia ruckeri*. The evaluation of the *in vitro* activity of oxolinic acid against these two bacteria was lower or equal to 0.10 µg/ml.

The compound administered in the feed under standard breeding conditions at a dosage-level of 12 mg/kg/day, enabled to maintain serum and tissue levels which were compatible with the MIC of the pathogens responsible for the two diseases mentioned above.

On the whole, the homogenous behaviour of oxolinic acid both in the serum and the tissues should also be noted. The present experimental conditions, which were comparable with those of the field practice, made it impossible for us to verify the feed intake and to ensure that the prescribed dose-level (12 mg/kg/day for 7 consecutive days) was actually ingested by the fish.
The method of analysis used in this study was perfectly reliable at concentrations of 10 ppb oxolinic acid, thus confirming the preponderant role played by HPLC for the determination of drug traces.

Considering the commercial destination of the treated fish, the interruption of the treatment must be followed by a rapid oxolinic acid residue elimination from the tissues. Under the experimental conditions used in our study, a tolerance level of 0.05 ppm was reached around the sixth day after withdrawal of the treatment. In the meantime, the elimination was still taking place, and a value of 0.03 ppm was achieved 10 days after the last administration.

In conclusion, taking into account the work published in the last decade concerning the fish farming industry and the results of our bioavailability study, the supplementation of oxolinic acid in the fish feed can be considered as an interesting alternative for the treatment of furunculosis and yersiniosis in farmed trouts.

Acknowledgements

We wish to thank Monsieur Kermes, the President of the « Federation Départementale des Associations de Pêche et Pisciculture » and Monsieur Guigo for their kind cooperation.

Abstract

A study was performed on the serum bioavailability and tissue elimination of oxolinic acid in the trout. The compound was added to the diet and administered at a dosage-level of 12 mg/kg/day for 7 consecutive days. The study utilized an analytical technique, high performance liquid chromatography, which has been described in detail here. The results obtained demonstrate that serum concentrations higher than the MIC for the control of the target pathogens (Aeromonas and Yersinia) can be sustained throughout the treatment period. The same positive results were observed in the tissues. Besides, on the base of a tolerance level of 0.05 ppm for the residue levels of oxolinic acid in the edible tissues (muscle mass and skin), a withdrawal time of six days after interruption of the prescribed treatment can be proposed.

References


