養殖ブリの類結節症に対する微細化オキソリン酸の効果

誌名	日本水産學會誌
ISSN	00215392
著者	高橋, 幸則 遠藤, 俊夫
巻/号	53巻12号
掲載ページ	p. 2157-2162
発行年月	1987年12月

Nippon Suisan Gakkaishi 53(12), 2157-2162 (1987)

Evaluation of the Efficacy of an Ultrafine Preparation of Oxolinic Acid in the Treatment of Pseudotuberculosis in Yellowtail

Yukinori Takahashi*1 and Toshio Endo*2

(Accepted July 29, 1987)

Antibacterial activity of oxolinic acid (OA) against *Pasteurella piscicida* isolated from yellowtail was studied, and field trials were carried out in order to compare the efficacy of ultrafine (1.0 μm in diameter) and ordinary (6.4 μm in diameter) preparations of OA against pseudotuberculosis in yellowtail.

The MIC_{90} values of OA, oxytetracycline, thiamphenicol and ampicillin against *P. piscicida* were 2.66, 35.70, >100 and 32.98 μg/m*l*, respectively.

In the field studies, each of the above two preparations of OA was mixed into minced sand eel and administered in doses of 10, 20 and 30 mg/kg of the ultrafine preparation and in a dose of 30 mg/kg of the ordinary preparation for 5 days after the onset of pseudotuberculosis outbreak. Both preparations reduced the mortality of the fish, and in group mortality, the ordinary preparation of OA at the dose of 30 mg/kg was intermediate between 10 and 20 mg/kg of the ultrafine preparation, when the mortality during and after the medication period and the mortality after the medication were both compared among the groups by statistical analysis.

Oxolinic acid (OA) is a compound having high antibacterial activity against gram-negative bacteria and certain gram-positive bacteria. In Japan, this compound has been widely used as a chemotherapeutic agent for bacterial infections in domesticated animals, fowls and cultured fish.[1-3] However, being sparingly soluble in water, OA has the disadvantage of limitations on methods of use and accordingly finely divided OA with a reduced particle diameter (ultrafine OA) has been developed and shown to assure improved bioavailability in beagle dogs, red sea-bream, and yellowtail.[4-6] To clarify the therapeutic effect of ultrafine OA on pseudotuberculosis in cultured yellowtail, a field study using the conventional coarse-particle OA as a control was conducted. The sensitivity of the *Pasteurella piscicida* strains isolated from culture yellowtail in 1986 to various antibacterial agents was also investigated.

Materials and Methods

Sensitivity Study

Test antibacterial agents: Oxolinic acid (OA), ampicillin sodium (ABPC), oxytetracycline hydrochloride (OTC) and thiamphenicol aminoacetate hydrochloride (TP) were tested.

Test organisms: 183 strains of *P. piscicida* isolated from yellowtail *Seriola quinqueradiata* associate with pseudotuberculosis on fish farms in the prefectures of Mie, Hyogo, Kochi, Ehime, Ohita, Miyazaki, Nagasaki, and Kagoshima during June to August, 1986 were employed.

Sesitivity test: Using 2% NaCl-sensitivity assay bouillon (Nissui) for multiplication and 2% NaCl-disk assay medium N (Nissui) for sensitivity determination, the susceptibility of each test strain to antibacterial agents was investigated by the MIC determination method established by Japan Society of Chemotherapy.[7] MIC_{90} were represented as the concentration of antibacterial agent required to inhibit at least 90% of the organisms. The inoculum size was 10^6 cells/m*l* and the cultivation was carried out at 25°C for 24 h.

Field Study

The field study was conducted in yellowtail which developed sign of pseudotuberculosis on Fish Farm A in Yamaguchi Prefecture in August, 1986.

*1 Department of Aquaculture and Biology, Shimonoseki University of Fisheries, Shimonoseki, Yamaguchi 759-65, Japan (高橋幸則: 水産大学校増殖学科).

*2 Biological Research Laboratory, Tanabe Seiyaku Co., Ltd., Toda, Saitama 335, Japan (遠藤俊夫: 田辺製薬株式会社生物研究所).

Test antibacterials: A 5% suspension of ultrafine OA with a particle diameter of 1.0 μm (TO-77S, Production No. 2Z001 Tanabe) and, as a reference control, a commercial oxolinic acid preparation containing 5% of OA crystals with a particle diameter of 6.4 μm (ordinary OA) (Parazan for acquaculture, briefly F-PAR, Production No. 57058 Tanabe) were used.

Test fish: About 76,000 yellowtail (mean body weight: about 60 g) which were caught in the same area and delivered to the above fish farm on July 20, 1986 were used.

Medication: The test fish were distributed in 13 net cages ($10\times10\times7$ m^3; No. 1 to No. 13), containing about 5,800 individuals per cage. After confirmation of outbreak of pseudotuberculosis, TO-77S mixed with minced sand eel *Ammodytes personatus* was administered in the following doses once a day for 5 days (August 9 to 13, 1986): TO-77S 30 mg (as OA/kg body weight; the same applies hereinafter) for Nos. 1 to 3 cages, 20 mg for Nos. 4 to 6 cages, 10 mg for Nos. 7 to 9 cages, and F-PAR 30 mg (as OA) for Nos. 10 and 11 cages. The fish in Nos. 12 and 13 cages were given drug-free minced sand eel as the untreated control. The ration was the same for all groups, *i.e.* 40% till day 2 after initiation of the experiment, 30% for day 3 through day 13, and 25% for day 14 through day 24.

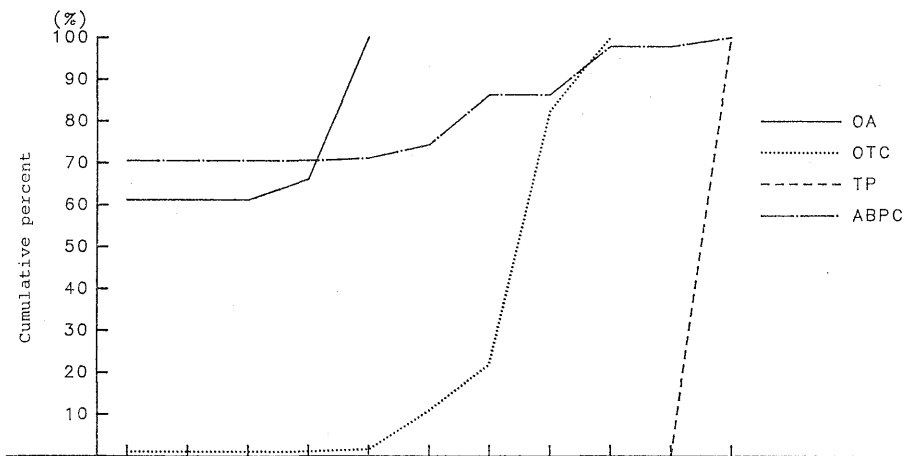
Observation of test fish and bacteriological examination: During the 21-day period from the 7th to 27th, August, 1986, the number of dead fish in each cage was recorded daily. About 100 fish were sampled at random and weighed on day 1 of the experiment (2 days before initiation of treatment) and on day 24 of the experiment (day 18 after completion of medication). On day 1 of the experiment, day 8 (day 1 after completion of medication) and day 21 (day 14 after completion of medication), 5 to 10 fish, either moribund or immediately after death, were sampled and autopsied, and using 2% NaCl-brain heart infusion-agar medium (Eiken), the isolation of bacteria was attempted from the heart blood, spleen, kidney and brain. After 48 h incubation at 25°C, the colony suspected to be *P. piscicida* was subjected to pure culture and investigated for Gram's stain, morphology, motility, oxidase, catalase, OF, gas production from glucose, sensitivity to O/129, slide agglutination using anti-*P. piscicida* serum, and so on for identification.

Further, the strains identified as *P. piscicida* were tested for sensitivity to OA using sensitivity disks containing 3 concentrations (10, 2 and 0.5 μg per disk) of OA.

Results

Sensitivity of P. piscicida Isolated from Yellowtail

The MIC distribution of 4 test antibacterial agents against 183 strains of *P. piscicida* isolated from cultured yellowtail associated with pseudo-



MIC(ug/ml)	=<.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	Total
OA	112*			9	62							183
OTC	2				1	17	20	111	32			183
TP											175	175
ABPC	129				1	6	22		21		4	183

Fig. 1. Antibacterial activity (MIC) of oxolinic acid and other antibacterial agents against *P. piscicida* isolated from yellowtail in 1986.
* Number of strains.

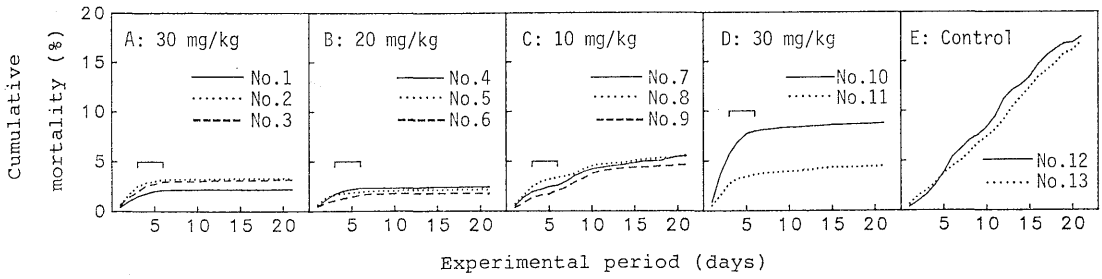


Fig. 2. Cumulative mortalities for the yellowtail treated with ultrafine (A-C) and ordinary (D) preparations of oxolinic acid at doses of 10 to 30 mg/kg. The fish (2 or 3 replicates per group) were given the drug-free diet for the first 2 days and then given the diet containing either the ultrafine preparation or the ordinary preparation of oxolinic acid for the subsequent 5 days. Each line represents the time course of the cumulative mortality for a replicate of the corresponding dosage group.

tuberculosis are shown in Fig. 1. The MIC of OA was distributed over the range of ≦0.2 to 3.13 μg/ml with peaks at ≦0.2 and 3.13 μg/ml. The MIC of OTC was distributed over the range of ≦0.2 to 50 μg/ml with peaks at ≦0.2 and 25 μg/ml. Thus, both antibacterial agents showed bimodal distributions of MIC. The MIC of ABPC was distributed over ≦0.2 to >100 μg/ml, with peaks at ≦0.2, 12.5 and 50 μg/ml, thus showing a trimodal distribution. The MIC of TP was invariably >100 μg/ml. The MIC_{90} values of OA, OTC, TP and ABPC against *P. piscicida* were 2.66, 35.70, >100 and 32.98 μg/ml, respectively. Thus, OA showed the highest antibacterial activity.

Field Study

Cumulative mortality: The cumulative mortality of yellowtail in each group is shown in Fig. 2. In the untreated control cages (Nos. 12 and 13), the number of deaths did not decrease during the experiment period and the final cumulative mortalities were 17.47% and 16.95%, respectively. In the ultrafine OA 30 mg and 20 mg groups, the number of deaths began to show marked decreases on day 4 or 5 and the cumulative mortality was 2.21 to 3.31% in the 30 mg group and 1.77 to 2.41% in the 20 mg group. In the ultrafine OA 10 mg group, the number of deaths decreased gradually and the cumulative mortality was 4.63 to 5.60%. As to the ordinary OA 30 mg group, No. 10 cage showed a larger number of deaths than the control group on day 1 of medication but began to show a gradual decrease on day 5 of medication and gave a final cumulative mortality of 8.79%. On the other hand, No. 11 cage showed substantially the same time course of mortality as the ultrafine OA 10 mg group and gave a cumulative mortality of 4.47%.

Using the cumulative mortality for the period from initiation or completion of medication to day 21 after initiation of the experiment, one-way analysis of variance was carried out with the antibacterial agents as factors and the fish groups as replicates. As a result, for both the period after initiation of medication and the period after completion of medication, significant difference ($p<0.05$) was found. Then, multiple comparison was made by Scheffe's method. The resultsof multiple comparison analysis for cumulative mortality after initiation of medication are shown in Table 1. Thus, significant difference ($p<0.05$) was found between the control group and each of the ordinary OA 30 mg group and ultrafine OA 10 mg, 20 mg and 30 mg groups, and between each of the ordinary OA 30 mg group and the ultrafine OA 10 mg group and each of the ultrafine OA 20 mg and 30 mg groups. The results of multiple comparison analysis for cumulative mortality after completion of medication are shown in Table 2. Thus, significant difference ($p<0.05$) was found between the control group and each of the ordinary OA 30 mg group and ultrafine OA 10 mg, 20 mg and 30 mg groups, and between the ultrafine OA 10 mg group and each of the ordinary OA 30 mg group and ultrafine OA 20 mg and 30 mg groups.

Mean body weight and weight gain: The mean body weight at initiation of the experiment and on day 24 after initiation of the experiment and the percent body weight gain during the intervening period are shown in Table 3. The percent body weight gain data were analyzed by comparing each replicate of the control group with the treatment group replicates which had shown mean body weights approximately equal to the control replicate mean body weight at initiation of the ex-

Table 1. Analysis of variance in cumulative mortality 19 days after the first administration

Source	DF	SS	MS	Fo
Drug	4	328.442	82.110	206.14*
Error	8	3.187	0.398	
Total	12	331.629		

Control	>*	>*	>*	>*
Ordinary OA 30 mg/kg		NS	>*	>*
Ultrafine OA 10 mg/kg			>*	>*
Ultrafine OA 20 mg/kg				NS
Ultrafine OA 30 mg/kg				

* $p<0.05$.
NS=Not significant.

Table 2. Analysis of variance in cumulative mortality 14 days after the last administration

Source	DF	SS	MS	Fo
Drug	4	207.213	51.803	1185.26*
Error	8	0.350	0.044	
Total	12	207.563		

Control	>*	>*	>*	>*
Ordinary OA 30 mg/kg		<*	NS	NS
Ultrafine OA 10 mg/kg			>*	>*
Ultrafine OA 20 mg/kg				NS
Ultrafine OA 30 mg/kg				

* $p<0.05$.
NS=Not significant.

Table 3. Body weight gain of yellowtail treated with the ultrafine and ordinary preparations of oxolinic acid

Group	Oxolinic acid	Dose (mg/kg)	Number of fish	Weight		
				0 day*1 (g)	24 days*2 (g)	Gain (%)
1	Ultrafine	30	5712	78	190	143.6
2			5858	91	185	103.3
3			5611	70	170	142.9
4		20	5817	62	180	190.3
5			5963	68	165	142.6
6			5916	52	155	198.1
7		10	5791	46	150	226.1
8			5931	54	160	196.3
9			5876	39	150	284.6
10	Ordinary	30	5940	39	170	335.9
11			6000	40	150	275.0
12	Control	0	5771	65	145	123.1
13			5682	71	150	118.3

*1 Two days before the first administration.
*2 18 days after the last administration.

periment. Thus, No. 12 (control) was compared with No. 4 and No. 5 (ultrafine OA 20 mg), and No. 13 (control) was compared with No. 3 (ultrafine OA 30 mg) and No. 5 (ultrafine O 20 A mg). The control group invariably showed lower body weight gains. Incidentally, throughout the experiment period, the test fish showed no abnormality ascribable to the administration of OA.

Isolation of bacteria: The isolation frequency of *P. piscicida* in each group is shown in Table 4. The isolation frequencies at intiation of the experiment (2 days before initiation of medication) were in the range of 80 to 100%, indicating that fish casualties on this farm were due to *P. piscicida*. The isolation frequency on day 8 after initiation of medication (1 day after completion of medication) was 0–20% in the ultrafine OA 30 mg group, 0% in the ultrafine OA 20 mg group, 20–40% in the ultrafine OA 10 mg group, and 80–100% in the untreated control group. The isolation frequencies on day 21 after initiation of the experiment (14th day after completion of medication) were also similar to those on day 8. Thus, a dose dependency was found. The isolation frequency in the ordinary OA 30 mg group was approximately equal to the frequency in the ultrafine 10 mg group. The disk assays for the OA sensitivity of *P. piscicida* strains isolated during the experi-

Table 4. Isolation of *P. piscicida* from test fish

Group	Oxolinic acid	Dose (mg/kg)	Presence of *P. piscicida*		
			0 day*1	8 days*2	21 days*3
1	Ultrafine	30	10/10	1/5	0/5
2			8/10	0/5	0/5
3			10/10	0/4	0/5
4		20	10/10	0/5	0/5
5			10/10	0/5	0/5
6			9/9	0/5	0/5
7		10	9/10	2/5	3/5
8			10/10	1/5	2/5
9			7/7	2/5	1/5
10	Ordinary	30	9/10	1/5	2/5
11			10/10	1/5	1/5
12	Control	0	10/10	4/5	5/5
13			10/10	5/5	5/5

*1 Two days before the first administration.
*2 One day after the last administration.
*3 14 days after the last administration.

ment showed ## for all strains.

Discussion

It has been shown in beagle dogs,[4] red seabream[5] and yellowtail[6] that the bioavailability of OA is increased as its particle size is reduced.

In the present study, 10, 20 and 30 mg of ultrafine OA and 30 mg of ordinary OA were orally administered to yellowtail for 5 days at the epizootic of pseudotuberculosis on a yellowtail farm in Yamaguchi Prefecture and an intergroup comparison was made for cumulative mortality during an 18-day period following commencement of medication. As a result, the mortalities in the ultrafine OA 20 mg and higher dose groups were significantly ($p<0.05$) lower than the mortalities in the ordinary OA 30 mg group. The reason why more than the equivalent efficacy was obtained with ultrafine OA in lower doses in comparison with ordinary OA seems to be the above-mentioned increased bioavailability of OA due to reduction of particle size. Therefore, as far as drugs which are sparingly soluble and less efficiently absorbed, such as ordinary OA, are concerned, it appears to be a worthwhile attempt to increase their bioavailability by reducing the particle size barring occurrence of side effects.

The intergroup comparison of the cumulative mortality during a 2-week period following completion of medication and the detection rate of *P. piscicida* during the same period revealed that while the ordinary OA 30 mg group gave a higher detection rate than the ultrafine OA 20 mg and 30 mg groups, no significant difference was found in cumulative mortality. On the other hand, the cumulative mortality was significantly ($p<0.05$) higher in the ultrafine OA 10 mg group than in the above 3 groups. These results suggest that the therapeutic efficacy of ordinary OA 30 mg is higher than that of ultrafine 10 mg OA and slightly lower than that of ultrafine OA 20 mg. Based on the above result, the detection rate of *P. piscicida* mentioned above, and the suggested maximum daily dosage of 30 mg/kg fish body weight for ordinary OA for the treatment of pseudotuberculosis in yellowtail,[8] it is tentatively concluded that the appropriate dosage of ultrafine OA for the treatment of pseudotuberculosis in yellowtail is 20 mg or more a day.

The *P. piscicida* strains isolated from morbid fish in this field study showed high sensitivity to OA but strains resistant to several chemotherapeutics other than OA are known to exist.[9] Therefore, the sensitivity of *P. piscicida* to antibacterial agents was investigated using 183 strains isolated from yellowtail associated with pseudotuberculosis in 1986. The MIC_{90} value of OA was found to be 2.66 μg/ml, indicating high antibacterial activity as compared with the reference antibacterial agents ABPC, OTC and TP. However, the bimodal pattern of MIC distribution indicated the emergence of strains with reduced sensitivity to OA. Under the circumstances, the

authors intend to conduct a detailed field investigation in order to clarify the efficacy of ultrafine OA in yellowtail affected by strains of *P. piscicida* with reduced susceptibility to OA.

References

1) T. Endo, K. Ogishima, H. Hayasaka, S. Kaneko, and S. Ohshima: *Nippon Suisan Gakkaishi*, **39**, 165–171 (1973).
2) B. Austin, J. Rayment, and D. J. Alderman: *Aquaculture*, **31**, 101–108 (1983).
3) H. Tagata, K. Hamada, K. Fukushima, E. Hashimoto, S. Fukunaga, S. Fujiyama, T. Yamashita, K. Ishizu, and F. Saigo: *Chikusan-no-Kenkyu*, **35**, 371–378 (1981).
4) Y. Hirakawa and K. Harada: *YAKUGAKU ZASSHI*, **103**, 1190–1194 (1983).
5) T. Endo, M. Onozawa, M. Hamaguchi, and R. Kusuda: *Nippon Suisan Gakkaishi*, **53**, 1493 (1987).
6) T. Endo, M. Onozawa, M. Hamaguchi, and R. Kusuda: *Nippon Suisan Gakkaishi*, **53**, 1711–1716 (1987).
7) Committee for Revision of MIC Determination Method: Chemotherapy, **29**, 76–79 (1981).
8) S. Yasumoto, N. Yasunaga, and K. Hatai: *Bull. Nagasaki Pref. Inst. Fish.*, No. 10, 71–78 (1984).
9) N. Takashima, T. Aoki, and T. Kitao: *Fish Pathology*, **20**, 209–217 (1985).