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Source: Journal of Wildlife Diseases, 24(1) : 22-24

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-24.1.22

Journal of Wildlife Diseases. 24(1), 1988, pp. 22–24

EFFECTS OF THE ANTIMICROBIC TIAMULIN ON SEVEN GRAM-NEGATIVE BACTERIAL FISH PATHOGENS

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ABSTRACT: In vitro and in vivo tests were carried out with tiamulin and gram-negative bacterial pathogens of fish. Determination of minimum inhibitory concentration for 51 strains of seven species of gram-negative bacterial pathogens showed that only strains of *Vibrio anguillarum* were sensitive at 1.6–6.25 ppm, while the rest of test strains required 25–>100 ppm. Control of infection was not achieved when tiamulin was fed for 14 days at 5 or 50 mg/kg to rainbow trout (*Salmo gairdneri*) experimentally infected with *Yersinia ruckeri*.

Key words: Tiamulin, gram-negative pathogens, disease, fish, antibacterial, experimental testing, pharmacology.

INTRODUCTION

The in vitro and in vivo testing by Bosse and Post (1983) of 15 antibacterial drugs for control of enteric redmouth disease indicated that two were the most effective: tiamulin (a semi-synthetic derivative of pleuromulin produced by a basidiomycete) and Tribrissen® (a potentiated sulfonamide). We undertook further testing of tiamulin as part of the research of the U.S. Fish and Wildlife Service in support of registration of chemicals and antibacterials for use in controlling diseases in cultured food fish species. We did not include Tribrissen® because another potentiated sulfonamide, Romet has been registered recently with the Food and Drug Administration for treatment of fish furunculosis, and extension of the registration to include enteric redmouth disease is anticipated.

MATERIALS AND METHODS

The minimum inhibitory concentration (MIC) of tiamulin was determined for several strains of each of seven gram-negative bacterial fish pathogens: *Yersinia ruckeri* (13 strains), *Aeromonas salmonicida* (12 strains), *A. hydrophila* (six strains), *Pseudomonas fluorescens* (six strains), *Edwardsiella tarda* (four strains), *E. ictaluri* (five strains), and *Vibrio anguillarum* (five strains). All test cultures were from the culture collection of this laboratory and were isolated from diseased fish. Test concentrations of tiamulin were prepared by dissolving 0.2 g of 100% active tiamulin (SDS Biotech Corporation, Painesville, Ohio 44077, USA) in 20 ml deionized water. The resulting 10,000 ppm stock solution was filtered through a membrane of 0.45 μm mean porosity (Millipore Corporation, Bedford, Massachusetts 01730, USA) and diluted in Mueller Hinton broth (Difco Laboratories, Detroit, Michigan 48232, USA) to provide final concentrations of 100, 25, 6.25, 1.6, 0.4, 0.1, and 0.025 ppm. The test concentrations were prepared in sterile 100-ml flasks and 5-ml portions were aseptically distributed to sterile test tubes (16 × 125 mm). All cultures were grown in Mueller Hinton broth for 24 hr at 25 C; they were diluted 1:100 in the broth and 0.1 ml of this diluted culture was used to inoculate each test concentration of tiamulin, as well as a tube of the broth (to verify viability of inocula). All tubes were incubated at 25 C and growth was recorded at 24 and 48 hr.

An in vivo trial was conducted to assess the potency of tiamulin in controlling disease in rainbow trout (*Salmo gairdneri*) experimentally infected with *Y. ruckeri*. We transferred about 60 (500 g) rainbow trout fingerlings that were raised at this laboratory (mean weight 2–3 g) to each of 12 tanks (38 liters) supplied with spring-water at 12.5 C, delivered at the rate of 1 liter/min. The trout were challenged for 60 sec in 2 liters of a 24-hr brain heart infusion broth (Difco Laboratories, Detroit, Michigan 48232, USA) culture of *Y. ruckeri*. Water was drained immediately before addition of the culture and the flow was resumed after challenge. Sixty trout in triplicate tanks were fed a diet containing 5 or 50 mg/kg tiamulin or 50 mg/kg Romet (Hoffman LaRoche Company, Inc., Nutley, New Jersey 07110, USA). Trout in triplicate tanks, fed a nonmedicated diet, were the controls. Vegetable oil (Procter and Gamble, Cincinnati, Ohio 45202, USA) was used as a binder (Bullock et al., 1983) to coat tiamulin and Romet onto the pelleted trout feed (Federal formula GR-6). The medicated diets were fed twice daily, beginning

TABLE 1. Minimum inhibitory concentration of tiamulin for seven gram-negative fish pathogens.

Test organism	Number of test strains	ppm Tiamulin				
		>100	100	25	6.25	1.6
Aeromonas hydrophila	6	3[a]	3	0	0	0
Aeromonas salmonicida	12	0	6	5	1	0
Edwardsiella ictaluri	5	0	2	2	1	0
Edwardsiella tarda	4	0	2	2	0	0
Pseudomonas fluorescens	6	5	1	0	0	0
Vibrio anguillarum	5	0	0	0	4	1
Yersinia ruckeri	13	5	7	1	0	0

[a] Number of strains showing inhibition.

2 days before the challenge and continuing for a total of 14 days. Mortality was recorded daily for 21 days after the exposure, and we confirmed the presence of *Y. ruckeri* in representative samples of the fish that died by using a direct fluorescent antibody test (Bullock et al., 1980).

RESULTS AND DISCUSSION

Of the seven species of bacteria tested, six showed a rather uniform resistance to tiamulin, but strains of *Vibrio anguillarum* were sensitive at an MIC range of 1.6–6.25 ppm (Table 1). In the 51 strains of the seven species, the MIC was >100 ppm in 13 (25%); 100 ppm in 21 (41%); 25 ppm in 10 (20%); 6.25 ppm in six (12%); and 1.6 ppm in one (2%).

Regardless of the dosage rate and the fact that the feeding of tiamulin was begun 2 days before trout were challenged, mortality in the treated and control groups was essentially the same. Mortality from the replicates 21 days postexposure was 60, 47, and 27 (134 of 200 or 67%) in the 5 mg/kg group; 45, 44, and 20 (109 of 165 or 66%) in the 50 mg/kg group; and 52, 27, and 45 (124 of 198 or 63%) in nonmedicated controls. There was no mortality in trout fed the 50 mg/kg dosage of Romet for 5 days. Although mortality varied among replicates in the tiamulin treatment groups and nonmedicated controls, total mortality in these groups did not differ significantly (chi-square = 1.73, df = 3, $P = 0.6$).

Thus, our results failed to confirm the finding of Bosse and Post (1983) that tiamulin controlled enteric redmouth disease. Only one of the 12 *Y. ruckeri* cultures was slightly sensitive to tiamulin (>6.25 ppm); MIC's in the remaining cultures ranged from 25 to >100 ppm. Similar results were obtained by DeGrandis and Stevenson (1985), who reported a range in MIC of 16–128 ppm for *Y. ruckeri*.

Results from the laboratory in vivo test supported the in vitro sensitivity studies. When tiamulin was fed for 14 days at the recommended daily level of 5 or 50 mg/kg, 10 times the level recommended by Bosse and Post (1983), the mortality was no different from that in nonmedicated controls. There was no mortality in the rainbow trout that received a 5-day treatment of Romet.

The in vitro sensitivities of test strains of *A. salmonicida*, *A. hydrophila*, *E. tarda*, *P. fluorescens*, and *E. ictaluri* indicated that tiamulin would probably not be effective in controlling epizootics caused by these pathogens. In 24 of the 26 strains, the MIC was >6.25 ppm.

The in vitro and in vivo tests strongly indicated that tiamulin has limited (if any) use in treating epizootics in fish caused by gram-negative pathogens. These results are consistent with information listed in the Merck Veterinary Manual (Fraser, 1986), which indicates that tiamulin is active mainly against gram-positive bacteria.

ACKNOWLEDGMENTS

We thank Darlene Bowling for technical assistance during the study, and the SDS Biotech

Corporation, Painesville, Ohio for supplying tiamulin. Use of trade names does not imply U.S. Government endorsement of commercial products.

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Received for publication 15 April 1987.

Journal of Wildlife Diseases, 24(1), 1988, p. 24

BOOK REVIEW . . .

Primates: The Road to Self-Sustaining Populations, Kurt Benirschke (ed.). Springer-Verlag, 275 Fifth Ave., New York, New York 10010, USA. 1986. 1044 pp. $79.00 U.S.

This book represents the proceedings of the international conference on wildlife conservation held in June 1985 at San Diego, California. The quality and evenness of the chapters are generally excellent; they are well written and easy to read. Most are thoroughly referenced in addition to being supplemented with information tables and charts.

The purpose of the conference was not only to arouse more widespread concern about the situation of the world's primate populations but also to foster interdisciplinary communication among scientists and field researchers in order to stem the approaching tide of primate species extinction.

The book represents contributions from field scientists, primatologists, behavioralists, reproductive specialists, virologists, and others. The volume generally follows the theme of the meeting, beginning with a review by field scientists of the status of primates in their natural ecosystems, and problems they face in their natural contexts from conservation programs. This review is followed by extensive contributions from those specializing in the management and propagation of captive primate populations. Specific consideration is given to reproductive phenomena, infection, and other basic scientific contributions such as pathology and genetics.

This volume will serve as a uniquely useful resource to students and professional conservationists owing to the diversity of the chapters. Many topics that would ordinarily be difficult to locate in the general literature because of their special nature, such as the preparation of specimens for viral isolation or management strategy for captive chimpanzee populations, are described here. In addition, because of the multidisciplinary nature of the book, it is interesting reading for the wildlife scientist. The emphasis of the text is primarily on technological intervention; however, as several contributors point out, the ultimate solution for the long-term conservation of primate species lies in the emphasis on the preservation of species in their natural ecosystems. In spite of the unresolved issues, this book is a valuable contribution to the literature of wildlife conservation.

The dialogue generated by this conference is exciting and thought provoking. Although there is no simple way to "build a road to self-sustaining populations," the conference and this book represent a positive contribution to worldwide primate conservation.

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