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BATH TREATMENT OF CHANNEL CATFISH WITH THREE BROAD-SPECTRUM ANTIBIOTICS

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Abstract: Serum drug levels were measured in channel catfish following bath exposure to kanamycin, gentamicin and chloramphenicol. Kanamycin was absorbed at a rate sufficient to attain therapeutic blood levels in several treatment schedules. Therapeutic blood concentrations could not be attained with gentamicin or chloramphenicol following 24 hrs exposure at 80 and 100 μg/ml water concentrations, respectively.

INTRODUCTION

Chemotherapy of the bacterial diseases affecting fish has been attempted by administering antibiotics and other chemical agents in the feed, by injection, and by incorporation of the agents into a bath.[13] Oxytetracycline,[9] chloramphenicol[10] and some sulfonamides[5] have been demonstrated as efficacious for treatment of systemic infections when incorporated into feed. However, when fish are anoretic due to disease, stress conditions or natural phenomena such as observed in adult salmon,[1] medicated feeds are of no value. Dosage problems also can be encountered with medicated feeds because environmental temperature changes affect food consumption or because of leaching of the antibiotic from the feed in water. While parenteral administration of antimicrobial agents in fish assures the proper dosage to all individuals, it requires time-consuming handling of the fish and is not economically feasible unless the fish are very valuable or only a few are involved. Handling may also mechanically damage the skin or spread infections by contact contamination with the handling equipment. Bath treatments with antibiotics have been used almost exclusively for control of superficial infections.[7]

Aeromonas, Vibrio and *Pseudomonas* species of bacteria are frequently encountered as fish pathogens.[12,13] Antibiotic sensitivity tests have been performed on many virulent *Aeromonas* and *Vibrio* isolates from fish by the Department of Veterinary Microbiology, Texas A & M University.[8] These data indicate that 90% of 50 isolates tested were sensitive to kanamycin and chloramphenicol and that all isolates were sensitive to gentamicin.

Kanamycin and gentamicin are aminoglycoside antibiotics which interfere with bacterial protein synthesis and are bacteriocidal in their effect on sensitive organisms. They are absorbed only poorly through the mammalian gastrointestinal tract and are usually administered parenterally. The growth of a broad range of Gram-negative bacilli is inhibited by gentamicin at 5.0 μg/ml or less.[14] Kanamycin inhibits several *Aeromonas* and *Pseudomonas* sp. at 1.6-3 μg/ml and can elicit its bacteriological effect at 3.1-12.5 μg/ml.[2] Clinical trials with kanamycin in fish have shown it to be effective in treatment of some bacteria diseases.[3,4] Chloramphenicol is a p-nitro-benzaldehyde derivative which also interrupts bacterial protein synthesis but the effect is bacteriostatic. This agent is absorbed well from the alimentary tract of terrestrial

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animals. Most Gram-negative bacteria are inhibited by 1 μg/ml or less,[11] but pseudomonads are resistant to chloramphenicol therapy. In addition to the Gram-negaitve bacterial pathogens usually associated with fish diseases, other virulent Gram-negative and Gram-positive organisms are sensitive to these three broad-spectrum antibiotics.

In this study catfish were exposed to kanamycin, gentamicin and chloramphenicol in bath treatments to determine if the antibiotics might be absorbed at a rate sufficient to attain therapeutic blood levels.

METHODS

Channel catfish (*Ictalurus punctatus*), 15 to 25 cm, that had no previous exposure to any antimicrobial agents were used in these tests. Blood was collected from the caudal artery by needle and syringe without anticoagulants. Serum samples from a minimum of 10 fish were pooled for each time-concentration determination. All tests were conducted at 23 to 25 C in water at a pH range of 7.8 to 8.2. Water in the exposure tanks was aerated and circulated but not filtered during the test periods.

Chloramphenicol[4] was assayed using a cylinder-plate technique similar to that described by Grove and Randall.[6] *Sarcina lutea* ATCC #15220 was used as the test organism as it provided needed sensitivity. The minimum detectble concentration of chloramphenicol was 0.5 μg/ml.

Gentamicin sulfate[5] determinations also were made with a cylinder-plate procedure using *Bacillus subtilis* ATCC #6633 for detection of levels to 0.4 μg/ml, and *Staphylococcus aureus* ATCC #6538P for serum concentrations of 0.5 to 2.0 μg/ml. The test sensitivity of free gentamicin was 0.02 μg/ml with this procedure.

The cylinder-plate assay for kanamycin sulfate[6] utilized *B. subtilis* ATCC #6633. The lowest detectable level of kanamycin sulfate for this test was 0.1 μg/ml.

Stainless steel plates[7] with six wells were used in 100 x 15 mm petri plates in the procedures for each antibiotic. Three-tenths milliliter (0.3 ml) aliquots of standard dilutions of the antibiotics or serum samples were placed in alternate wells using not less than three plates (nine wells) for any mean zone diameter determination. After a 24 hr incubation, zone diameters were measured and averaged for each standard or sample. From these data a linear regression line was calculated and concentrations in the unknowns were determined from its equation.

As a screening procedure for uptake of the drugs, groups of ten fish were treated in baths of the individual antibiotics for 24 hrs and then sampled. The single antibiotic (kanamycin) which attained a therapeutic level under these conditions was further evaluated in the fish and the half-life of the drug in the water system was also determined.

Since therapeutic blood levels of kanamycin were reached in 24-hr exposures, shorter intervals were then tested by treating fish in kanamycin baths of 40 to 640 μg/ml for 2 hrs. The plasma clearance rate for the drug was determined in open and closed fresh water systems after the fish had been exposed to the antibiotic to establish a serum level (Figure 3).

RESULTS

Pooled sera were obtained from groups of 10 catfish after 24 hrs in each of three tanks with water concentrations of chloramphenicol of 20, 40 and 80 μg/ml. Chloramphenicol activity could not be detected ($< .5$ μg/ml) in any of the three pooled specimens. In the water system used in these studies, the activity of chloramphenicol in the bath decayed only 13.5% in 48 hrs.

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[4] Provided by Rachelle Laboratories, Inc., Long Beach, California 90801, USA.

[5] Provided by Schering Corporation, Bloomfield, New Jersey 07003.

[6] Provided by Bristol Laboratories, Syracuse, New York.

[7] Lab-Line Instruments, Inc., Melrose Park, Illinois 60160.

Catfish were exposed to gentamicin at water levels of 8, 24 and 100 μg/ml for 24 hrs. Gentamicin concentration was measured in the pooled serum samples from the three groups of 10 fish each at 0.05, 0.135 and 0.47 μg/ml, respectively.

Preliminary studies with kanamycin in a few fish indicated that therapeutic blood levels could be attained with the drug in 100 μg/ml within 24 hrs. The data obtained in a trial using 40 catfish (10 fish/sample time) in a 100 μg kanamycin/ml bath are presented in Figure 1. Fish in a 50 μg/ml bath had a mean serum level of 1.6 μg/ml at 24 hrs and the blood level continued to rise until a steady state was reached with the water at 48 hrs. The mean serum concentration at 48 hrs was 2.0 μg/ml.

The serum levels found in the fish after two hr exposures in bath concentrations of 40, 80, 320 and 640 μg/ml are plotted in Figure 2, demonstrating that therapeutically effective blood concentrations can be reached following as little as two hours exposure in a kanamycin bath.

With an initial mean kanamycin serum concentration of 2.0 μg/ml, fish placed into an open water system (complete water volume turnover twice a day) eliminated kanamycin at a rate to yield a serum decay half-life of 9.00 hrs (Figure 3). A similar study in a closed water system indicated a similar excretion rate (Figure 3). The half-life of free kanamycin in the closed water system was 4.5 days.

DISCUSSION

The uptake of chloramphenicol by catfish is not adequate to establish a therapeutic level of the free drug in the plasma when added to the water at 80 μg/ml. Nechiporenco *et al.*[10] observed that infectious dropsy could not be effectively treated with a chloramphenicol bath at

FIGURE 1

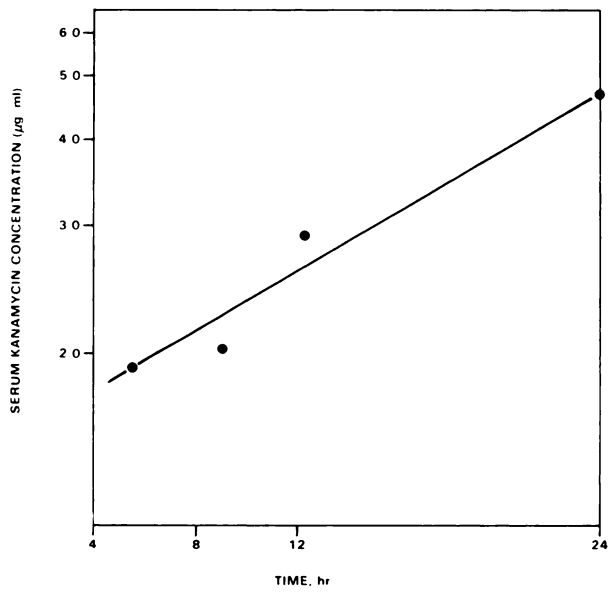

FIGURE 1. Serum levels of kanamycin in catfish following continuous exposure to the antibiotic in water (100 μg/ml) for 24 hrs.

FIGURE 2

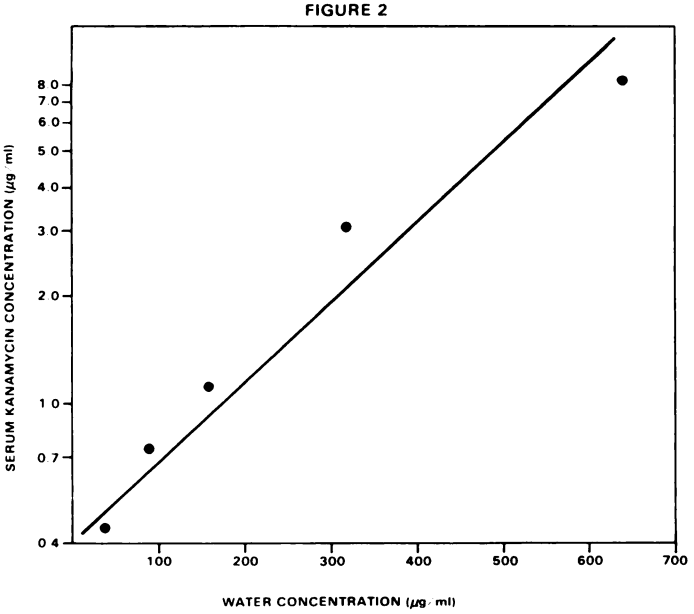

FIGURE 2. Kanamycin concentration in catfish serum following 2 hr exposure to the drug at various levels in water.

FIGURE 3

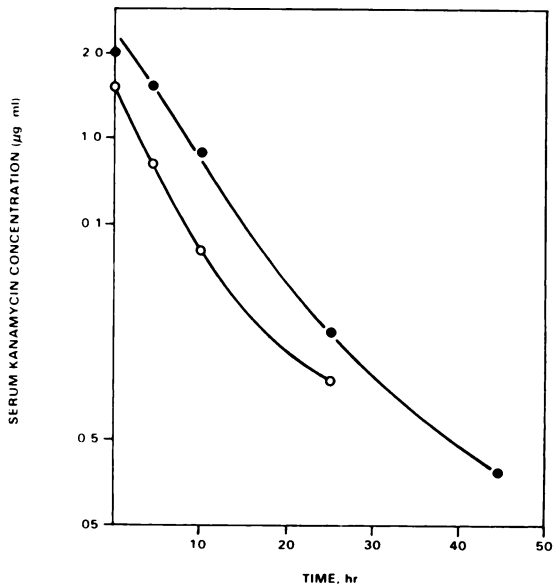

FIGURE 3. Elimination of kanamycin from the serum of catfish following removal from treated water (o: decay in open water system; x: decay in closed water system).

1000 μg/ml. The disease could be controlled if the drug was administered orally to the fish at 1 g/kg food. These observations indicate that bath treatments with chloramphenicol are of little value for treating systemic infections, such baths could be considered for control of superficial infections by susceptible pathogens.

Although gentamicin possesses a broad range of antimicrobial activity against bacterial fish pathogens, therapeutically effective plasma levels are not produced in catfish after 24 hrs exposure to a water concentration of 100 μg/ml.

Kanamycin was absorbed at a rate sufficient to establish therapeutic blood levels in 2 to 24 hrs depending upon the bath concentration. Effective systemic therapy should be possible therefore using kanamycin in a bath treatment procedure against susceptible bacteria.

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LITERATURE CITED

1. AMEND, D. F. and J. L. FRYER. 1968. The Administration of Sulfonamide Drugs to Adult Salmon. Progr. Fish-Cult. 30: 168-172.
2. CONROY, D. A. 1961. Estudio in Vitro de la Accion de la Kanamicina Sobre Bacterias Pathogenas Para los Peces. Microbiol. Espan. 14: 147-155.
3. ————. 1961. Las causas de un brote de putrefaccion de la aleta caudal en peces y su tratamiento con kanamicina. Microbiol. Espan. 14: 239-246.
4. ————. 1963. Studies on the application of kanamycin to the control and treatment of some bacterial diseases of fish. J. Applied Bact. 26: 182-192.
5. FLAKAS, K. G. 1948. Sulfonamide therapy of furunculosis in Brown Trout. Trans. Amer. Fish. Soc. 78: 117-127.
6. GROVE, D. C. and W. A. RANDALL. 1965. *Assay Methods of Antibiotics.* Medical Encyclopedia Inc., N.Y.
7. HERMAN, R. L. 1970. Chemotherapy of Fish Diseases: A Review. J. Wildl. Dis. 6: 31-34.
8. LEWIS, D. L. Unpublished data.
9. MEYER, F. P. 1964. Field treatments of *Aeromonas liquefaciens* infections in Golden Shiners (*Notemigonus crysoleucas*). Prog. Fish Cult. 26: 33-35.
10. NECHIPORENCO, YU, D., E. F. OSAACHAYA, I. M. KARPENKO and A. V. MAREEVA. 1962. Primenie levomitsetina diya bor'by krasnukhoi karpa'. Rybnoe Khor. 38: 30-31.
11. SENECA, H. 1971. *Biological Basis of Chemotherapy of Infections and Infestations.* F. A. Davis Company, Philadelphia.
12. SNIESZKO, S. F. 1975. History and Present Status of Fish Diseases. J. Wildl. Dis. 11: 446-459.
13. VAN DUIJN, C. 1973. *Diseases of Fishes.* Iliffe Books, London.
14. WAITZ, J. A. and M. J. WEINSTEIN. 1969. Recent Microbiological Studies with Gentamicin. J. Infect. Dis. 119: 355-360.

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