9-1975

Buquinolate as a Preventive Drug to Control Microsporidosis in the Blue Crab

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Abstract

When administered a single meal containing spores of *Nosema michaelis* and buquinolate, specimens of the blue crab, *Callinectes sapidus*, were less likely to acquire spore-ridden muscle tissue than individuals not allowed the drug. Those crabs presented the drug 48 hr preceding or following the introduction of spores also exhibited minimal incidence of infection. Even after 2 mo, spores were not observed in the musculature of most crabs.

Introduction

Buquinolate prevented microsporidosis caused by *Nosema michaelis* in most specimens of the blue crab, *Callinectes sapidus*, fed infective spores. The need to prevent infections in the blue crab is especially pronounced when considering the economic potential for rearing the crab for the soft-shelled crab industry, presently an underexploited fishery (Overstreet and Cook, 1972). Infection occurs when one crab feeds on spores from an infected individual. After the spore is ingested, vegetative growth of the parasite ensues in hemocytes located in sinuses in the connective tissue surrounding the midgut, followed by sporogenesis in the sarcoplasm of the striated muscle cells (Weidner, 1970). Dissolution of both infected and adjacent uninfected muscle fibers is considerable and, under certain conditions, the host becomes immobilized (Overstreet and Weidner, 1974). Infected crabs die quickly when under severe stress.

Methods and Materials

Crabs used in the study were collected from Mississippi Sound where *N. michaelis* infected only one individual out of a few thousand examined. Since death often accompanies molting in rearing facilities, mature females, which rarely, if ever, molt, were utilized exclusively. Spores used to feed uninfected crabs came from infected *C. sapidus* in Lake Pontchartrain, Louisiana, where low rates of infection commonly existed. Because trials were conducted during widely separated periods, spores were collected and processed at different times. After being separated from host tissue using centrifugation and saline washes, the concentrated spores were aged in refrigerated distilled water for as long as 348 days before being fed to crabs in inoculated portions of fish approximately 3 mm^3. Because the crab shreds its food while ingesting it, no accurate means could be established to measure concentrations of the drug. Micronized buquinolate, ethyl 4-hydroxy-6, 7-diisobutoxy-3-quinoline-carboxylate lot no. CN 161 produced by the Norwich Pharmaceutical Company, was, because it is nearly insoluble in water, administered with food that had been rolled in it. After the drug was introduced, the crab's diet consisted of two or three feedings of untreated diced fish per week. Crabs always remained separated, two to a 25-by-35-cm divided plastic box. Five covered boxes and a force-flow-type filter incorporating charcoal and ground-up oyster shell constituted a column. Each test group was confined in one or two separate closed systems. For each system, except in the pilot test, two columns emptied water into a single collecting container from which a dual-purpose pump forced to the top boxes less than 350 liters/hr of water having a salinity of approximately 12 ppt. Water then flowed by gravity to the other boxes, with a 75-mm level continuously maintained by overflow-tubes. It was exchanged periodically. A pilot test utilized one submersible pump for each column, but that type pump proved less reliable than the open-air type. It was conducted using one column per test group. The temperature of the water could not be regulated at a specific level, but, during the warmer months, an air conditioner maintained temperatures considerably less than the seasonal values. At the beginning of a trial immediately following initial ingestion of spores by the crabs, the temperature of the water was increased to about 30°C for 2 hr and then allowed to cool. Controls consisted of two groups: crabs presented spores without drugs and crabs presented neither spores nor drugs. Since some crabs died early in the experiment, and their deaths could not necessarily be attributed to the microsporidan, only crabs surviving after the first recognized infection were used in computations. All groups in the last two trials initially contained 40 individuals.

Heavy mature infections appear conspicuous in live crabs because of the chalky opaque character of infected muscles seen through the integument at joints of the appendages. In this study, however, muscle tissue from near the base of the fifth walking leg and from the hindgut of crabs that died was examined using a compound microscope. Only after infections with spores were determined to be well established in many crabs, were the remaining crabs sacrificed and examined.

Results

The number of crabs infected and examined in each treatment group appears in Table l. In the first trial, running for 101 days, 71% of the control crabs were infected, compared to 13% of those administered buquinolate. Spores were first demonstrated at 25 days in the muscular tissue of the hindgut and at 36 days in the appendage muscle of dead crabs. Using more crabs in the second trial, lasting 29 days after the spores were presented, produced similar results. However, in the last trial, which lasted 51 days, the outcome was the same, but all groups had fewer infected crabs than in the previous two trials. Spores used for the last trial had been aged nearly l yr compared with a much shorter time for the others. In the last two trials, the initial infection was noted 17 days after the spores were introduced.

Table 1. Percentage of Adult *Callinectes sapidus* Infected by *Nosema michaelis* With and Without Treatment with Buquinolate

Trial	I			II			III		
	Infected	Exam-ined	%	Infected	Exam-ined	%	Infected	Exam-ined	%
No drug, no spores				0	35	0.0	0	35	0.0
No drug, spores	5	7	71.4	24	38	63.2	11	36	30.6
Drug and spores simultaneously	1	8	12.5	7	32	21.9	0	36	0.0
Drug 48 hr preceding spores							2	33	6.1
Drug 48 hr following spores							1	33	3.0

Because the number of infected crabs in each of the columns comprising a treatment group reflected similar rates of infection, all the crabs in one such group were combined. Spores usually infected muscles from both appendages and the intestine, but occasionally they were noted in either one or the other.

Discussion

Buquinolate unquestionably caused a reduction in the number of crabs developing spore-ridden muscle tissue, even if applied 2 days preceding or following presentation of spores. Perhaps, by administering more than a single dose, the disease would not become manifest in any crabs experimentally infected and held under conditions reported in this paper. The dose necessary to prevent the disease, the stage of the parasite affected, and the mechanism involved were not studied.

In contrast to the finding of Weidner (1970), sporogenesis did occur in intestinal muscles of the infected crabs.

My finding that in the trials continuing for about 2 and 3 mo fewer crabs given the drug developed spores in their muscles than those in the trial lasting 29 days does not support

the presence of a long-lasting latent infection in the hemocytes. If hemocytes contained trophozoites inhibited by the action of buquinolate, I would expect to find heavy infections in the muscle after a reasonable length of time.

The drug, incorporated as the active ingredient in Bonaid, prevents coccidiosis in broiler chickens. As shown by the activity of buquinolate on the development of *Eimeria tenella* in embryonic chicken kidney cells, there is an inhibition or delay, depending on the concentration of the drug used, in the development of second-generation schizonts (McDougald and Galloway, 1973). In the chick, only the sporozoite when it first invades the host cell is susceptible to inhibition because the drug cannot penetrate deep enough into the gut wall to affect later stages (Ryley, 1967).

Variation in the number of crabs with discernible microsporidosis in the last two trials could be a result of the age or other factors determining viability of the spores, the temperature of the water or other seasonal variables, the state of the crabs, or other factors. Studies delimiting some of these factors plus the effects of repeated introduction with spores or drug are planned. Charles A. Johnson of Duke University Marine Laboratory has infected zoea through adult stages, and his investigations should show the relationship among the rate of infection and the crab's size and the water's temperature and salinity. He (Johnson, pers. comm.) always obtained a frequency of infection less than 10% in young and adult stages, even though he achieved higher rates in zoea and megalops. The raised temperature of circulating water for a short period following presentation of spores or the viability of spores may account for the higher frequencies I report.

Several other drugs were tested in addition to buquinolate with less success, and these, as well as some of the problems encountered during the study, will be reported separately. Mention of two of the drugs, however, seems relevant because the two have been found effective against other microsporidans.

Beekeepers consider bicyclohexylammonium fumagillin (2,4,6,8-decatetraenedioic acid 4-[l ,2epoxy-l, 5dimethyl-4-hexenyl]-5-methoxy-l-oxaspiro[2,5] oct-6-yl ester) the drug of choice to control *Nosema apis* in honeybees. Katznelson and Jamieson (1952) first demonstrated its use. The drug is also employed to control *Perezia pyraustae* in the European corn borer (Lynch and Lewis, 1971). A single dose of Fumidil B, the water-soluble form of the antibiotic produced by Abbott Laboratories, resulted in a slightly larger percentage of crabs becoming infected with *N. michaelis* than for the controls. One reason the drug may not be effective is that vegetative production of *N. apis* occurs in the intestinal cells, whereas in *N. michaelis* this stage exists in hemocytes. Jaronski (1972) demonstrated histochemically that Fumidil B inhibited RNA synthesis in the microsporidan *Octosporea muscaedomesticae* in the black blowfly. He also pointed out that in one fly the organisms in the apical, but not the basal, regions of the host cells were affected by the drug, suggesting fumagillin had difficulty entering the cell. Since vegetative growth of *N. michaelis* occurs in hemocytes, the inability of the drug to diffuse through the basal membrane of the gut epithelium, thereby not reaching the trophozoites, would explain the drug's inefficacy.

Benomyl, a systemic fungicide (methyl 1-[butylcarbamoyl]-2 benzimidazolecarbamate), was accidentally discovered by Hsiao and Hsiao (1973) to control *Nosema* sp. infecting the alfalfa weevil. In the concentration used for crabs, it was not nearly as effective as buquinolate on the microsporidan infection, and it apparently killed more crabs.

Synergism between drugs allows a much lower concentration of drugs to be used. Challey and Jeffers (1973) reported a drastic decrease in production of *Eimeria acervulina* Tyzzer oocysts in chicks given a combination of meticlorpindol and buquinolate than in those given either drug alone. This same drug, under the generic name clopidol (3,5-dichloro-2,6 dimethyl-4-pyridinol), did not enhance the effect of buquinolate on *N. michaelis*. In fact, in the only trial using the combination, of the fewer surviving crabs, more were infected than when buquinolate was used alone.

Note

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