Pathogenicity of a Birnavirus to Hard Clam (Meretrix lusoria) and Effect of Temperature Stress on Its Virulence

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A birnavirus, CV-TS-1 strain isolated from cultured hard clam (Meretrix lusoria) was inoculated to the hard clam by means of intrapallial cavity injection or water borne method at 25°C. Cumulative mortalities of clams injected with the virus at the concentrations of $10^{4.7}$, $10^{3.7}$ and $10^{2.7}$ TCID$_{50}$/clam were 37.5%, 32.5% and 25%, and those of clams immersed to virus solutions of $10^{6.0}$, $10^{5.0}$ and $10^{4.0}$ TCID$_{50}$/ml were 30%, 25% and 15%, respectively. Temperature stress (increase from 25°C to 33°C or decrease from 25°C to 15°C) was given to clams before and after virus challenge by exposure to a virus solution of $10^{6.0}$ TCID$_{50}$/ml. Mortality increased markedly when temperature was increased after virus infection, though either the decrease in temperature after virus challenge or increase or decrease in temperature before the challenge did not affect the mortality.

Introduction

Culture of the hard clam (Meretrix spp.) is economically important industry in Taiwan. Mass mortalities at clam farms which occur every March, June and September, have caused serious losses to the industry. Fluctuating temperatures, heavy rainfall, industrial pollution and diseases have been considered to be the possible cause for the outbreaks (Kou et al., 1984; Tseng, 1976, Yang et al., 1978). Many viruses have been isolated from marine bivalve molluscs and shown to be pathogenic to hosts (Dobos et al., 1979; Elston and Wilkinson, 1985; Farley et al., 1972; Meyers, 1979; Oprandy et al., 1981; Underwood et al., 1977). In Taiwan, several viruses have been isolated from cultured hard clams, including CV-HB-1, CV-TS-1, CV-TS-8 of the family Birnaviridae (Lo et al., 1988) and HCV of family Reoviridae (Hsu et al., personal communication). However, the pathogenicity of these viruses are not well clarified.

Environmental stressors such as water temperature and chemical pollutants, as well as biological factors like age or coinfection of other pathogens, were reported to have drastic effects on the pathogenicity of certain viruses (Dorson and Torchy, 1981; Farley et al., 1972; Hetrick et al., 1979). In the present study, attempts were made to study the pathogenicity of CV-TS-1 virus and the effect of water temperature on the susceptibility of clams to the viral infection.

Materials and Methods

Cells and Virus

CV-TS-1 virus, an aquatic birnavirus isolated from the gills of the clam Meretrix lusoria from a cultured farm near Tung-Shyr in southern Taiwan, was used for experimental infections in the present study. The virus was propagated in EO cell line which was derived from eel ovary by Chen and Kou (1981). The cells were cultured in Leibovitz's L-15 medium supplemented with antibiotics (penicillin 50 I.U./ml, streptomycin 50 μg/ml, fungizone 1.75 μg/ml). For routine passage or for virus propagation and titration, 5% or 2% fetal bovine serum (FBS) was supplemented to the medium respectively. The cells were incubated at 26°C.

Hard clam

Hard clams were obtained from a clam farm in Lu-Kang Prefecture of Taiwan, and were kept in sea water controlled at 25±1°C. They were fed a commercial powder diet once daily.
Infection trials

Infection trials were carried out by means of water borne inoculation and injection. For water borne inoculation, three virus concentrations of $10^{6.0}$, $10^{5.0}$ and $10^{4.0}$ TCID$_{50}$/ml were tested. Two replicates of thirty 4-month-old clams (mean weight 1.1 g) were immersed in virus solution for 2 h. For injection trials, virus suspension was injected at $10^{4.7}$, $10^{3.7}$ and $10^{2.7}$ TCID$_{50}$/clam into the pallial cavity of each clam. Two replicates of twenty 1-year-old clams (mean weight 5.6 g) were used for injection test. Control groups were either exposed to or injected with supernatant of EO cell culture which did not contain virus. After inoculation, clams were reared in running seawater controlled at $25 \pm 1^\circ$C with continuous aeration. The mortality was observed daily for 3 weeks.

Effect of temperature stress on the pathogenicity of CV-TS-1 virus

Temperature stress was applied to three sized hard clams (mean weight of 7.23 g, 4.35 g and 1.22 g, respectively) before or after exposure to CV-TS-1 virus suspension. Control groups were exposed to the supernatant of EO cell culture which did not contain virus. In Experiment I, clams were transferred from $25^\circ$C to $33^\circ$C or to $15^\circ$C and kept two days before they were exposed to virus solution of $10^{5.0}$ TCID$_{50}$/ml. After exposure to the virus solution, clams were reared in running seawater controlled at $25 \pm 1^\circ$C with continuous aeration. In Experiment II, clams were reared in seawater at $25^\circ$C for one week and exposed to the virus solution of $10^{5.0}$ TCID$_{50}$/ml for 2 h. After the exposure, temperature of the seawater was increased from $25^\circ$C to $33^\circ$C or decreased to $15^\circ$C in 2 h. The mortality was observed daily for 3 weeks.

Results

Infection trials

Cumulative mortalities of clams immersed in virus solutions of $10^{6.0}$, $10^{5.0}$, $10^{4.0}$ and 0 (control) TCID$_{50}$/ml were 30, 25, 15 and 0%, respectively (Fig. 1). No virus was reisolated from the survivors. In the present study the virus was not reisolated from dead clams, since it was difficult to know the death of the clams before their valves were opened. In addition when their valves were opened, no visceral mass were found due to autolysis. However, we reisolated virus from moribund clams in the preliminary experiment. In the experiment clams were immersed to virus solution and seven days after the infection, we opened the valves and could reisolate the virus from all unhealthy clams, which were thin and of which valves were easily opened.

Cumulative mortalities of clams injected with the virus solution at $10^{4.7}$, $10^{3.7}$, $10^{2.7}$ and 0 (control) TCID$_{50}$/clam were 37.5, 32.5, 25 and 2.5%, respectively (Fig. 2). No virus was re-isolated from the survivors though some clams showed gray gills.
Effect of temperature stress on the pathogenicity of CV-TS-I virus

Effect of temperature stress on pathogenicity of three sized groups of clams were shown in Figs. 3 and 4. As these figures show, the virus was pathogenic to the smallest clam tested and no mortality was observed in the other two groups of larger clams. Mortality of infected clams (mean weight of 1.22 g) reached 90% when temperature was increased after virus infection (Fig. 3). However, other treatments such as increase or decrease in water temperature before the virus challenge or decrease in water temperature after the challenge did not affect the mortality of the clams (Figs. 3 and 4).

Discussion

Pathogenicity of CV-TS-1 virus which was isolated from the abnormal dark gills of hard clams (Meretrix lusoria) from the Tung-Shyr area of Taiwan was studied. Cumulative mortalities of clams infected with virus by water borne exposure or by injection of the virus into the palleal cavity of experimental clams ranged from 15 to 40%. It appears that the virus is not a drastic pathogen to hard clams, but the present results suggest that the virus increases virulence to clams when the clam is exposed to the virus at an earlier age especially in some stressful conditions such as rapid change in ambient water temperature. Therefore, the virus seems potentially to possess a threat to the clam.

It is known that environmental stress conditions significantly increase the susceptibility of cultured organisms to diseases. Farley et al. (1972) indicated that higher mortality of oysters held at higher temperatures correlated well with the high prevalence of herpes-type virus inclusions in that group. Roberts and McKnight (1976) suggested that husbandry stress, such as transportation or sudden increase in temperature resulted in a recrudescence of infectious pancreatic necrosis (IPN). Castric et al. (1987) reported that mortality occurred when asymptomat-
Fig. 4. Cumulative mortalities of different sized hard clams infected by immersion of virus solution of $10^{5.0}$ TCID$_{50}$/ml and subsequently treated with temperature change. Group 1: 7.23 g; Group 2: 4.35 g; Group 3: 1.22 g. 

Contro; : Experiment. #: Virus infection; \(\nabla\): Temperature change.

ic turbots were transferred from 11°C to 18°C, and an IPN virus, serologically related to Ab serotype, was isolated.

Since 1969, the outbreaks of mass mortalities of clam in farms in Taiwan which occur every March, June and September, have been well documented (Tseng, 1976). Numerous reports have discussed the cause for them, and fluctuating temperatures, heavy rainfall, industrial pollution and diseases have been considered. However, few relate the occurrence of the mass mortalities with viral infection in combination with environmental stressors. The findings of this study indicate that the increase in temperature may have effects on the pathogenicity of CV-TS-1 virus. The relationship of stress and viral infection as the cause of mass mortality of hard clam in farms throughout Taiwan needs to be further clarified and evaluated.

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References


Dorson, M. and C. Torchy (1981): The influence of fish age and water temperature on mortalities of rainbow trout, Salmo gairdneri Richardson, caused by European strain of infectious pancreatic necrosis virus. J. Fish...


