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Carbaryl Inhibition of Interferon Synthesis in Cultured Goldfish Cells

Thomas B. Shea

Department of Biochemistry, E. K. Shriver Center, Waltham, MA 02254

Dose-response toxicity tests are commonly conducted to assess the impact of a pesticide on higher organisms, both in vivo and in vitro. However, in efforts to control such studies, environmental factors which could impart stress on the organism are often methodically excluded. The potential danger is that relatively common environmental conditions which stress a given organism may influence the toxicity levels of the pesticide in question.

Recent studies have shown carbaryl to be toxic to goldfish both in vitro (Shea and Berry 1983a) and in vivo (Shea and Berry 1983b). Though relatively short-lived in the environment, its major hydrolysis product, 1-naphthol, persists much longer (Steward et al. 1967); 1-naphthol is twice a toxic to goldfish in vitro, and 5 times more toxic in vivo, than its parent compound (Shea and Berry 1983a,b). Furthermore, 1-naphthol is taken up by goldfish cells in culture, and localizes in the nuclear fraction, leaving open the possibility of metabolic interaction. Given the stability of 1-naphthol in the environment, its localization in fish cells, the toxicity of both compounds to fish, the ability of both compounds to enhance fish virus replication (Shea 1983a,b), and the widespread use of carbaryl, an aquatic system contaminated by carbaryl runoff or mis-spraying is primed for the above mentioned synergistic interplay of pesticide and environmental stress.

In this report, carbaryl is shown to inhibit interferon synthesis by goldfish cells in vitro, at a concentration of carbaryl which does not induce any cytotoxicity nor alterations in growth rate. Consequently, while treated cultures are indistinguisable from controls, they are rendered more susceptible to viral infection by a subcytopathic concentration of carbaryl.

MATERIALS AND METHODS

Cultivation of the goldfish-derived CAR cell line (ATCC CCL-71) and its infection with Goldfish Virus-2 (GFV-2) have been previously described (Berry et al. 1983; Shea 1983a). Medium 199 was employed, supplemented with 10% fetal bovine serum, and 100 units each of penicillin and streptomycin, and 0.025 mcg of Fungizone/mL.

Cultures were treated with carbaryl as described previously

(Shea 1983a). Cells were plated in 25cm^2 flasks at a density of 10^5 cells/flask in medium without carbaryl. Twenty-four h later, this medium was replaced by medium containing 1 part per million (ppm) carbaryl (Technical grade, 99.07% pure, a gift of Union Carbide) obtained by serial dilution of a stock solution in 100% ethanol. The final concentration of ethanol in medium was 0.01%; previous studies have shown that 0.1% or less ethanol has no effect on CAR cells or GFV-2 replication (Shea and Berry 1982a, 1982b). Forty-eight h later this medium was removed and the cultures were infected with GFV-2 at a multiplicity of 1 in medium without carbaryl. Alternate cultures were harvested for determination of cell numbers, total protein/culture, microscopic examination for cytotoxicity, and determination of the number of cells/culture which were capable of adherence upon passage. The pH of culture medium was also determined.

To quantitate interferon activity (known to be produced by CAR cells in response to GFV-2 infection; ES Berry and TB Shea, manuscript in preparation), medium was aspirated from infected cultures, and uninfected controls, 5 d post infection, with and without pretreatment of these cultures with carbaryl prior to infection. This medium was centrifuged at 100,000 x G for 1 h to pellet cell debris and virus. The resulting supernatants were adjusted to pH 2 with HCL for 4 h (room temp) to inactivate residual virus and soluble capsid components, then readjusted to pH 7 with NaOH. The supernatants were diluted 1:4 with fresh medium and dispensed on to secondary CAR cultures in either 24-well trays (1 mL/well) or 96-well microtiter trays (0.1 mL/well), for quantitation of dye-binding or viral progeny synthesis, respectively (below). After 24 h this medium was removed and cultures were infected (multiplicity of 1) and incubated for 5 d. Biological activity of interferon was quantitated by two methods: (1) Comparison of the extent of gentian violet bound by infected cultures after pretreatment with various supernatants according to the interferon assay of Armstrong (1971); (2) comparison of the infectious titers of GFV-2 synthesized in CAR cells pretreated with various supernatants.

RESULTS AND DISCUSSION

Cultures treated with 1ppm carbaryl were indistinguishable from controls as assayed by phase microscopy, number of cells/culture, total protein/culture, or the percentage of cells surviving passage (Table 1). This is in agreement with previous studies in which 25ppm carbaryl were required to show marginal cytotoxicity after 7 d (Shea and Berry 1983a). A further indication of lack of drastic metabolic inhibition by carbaryl is the observation of equivalent pH values for treated and control cultures; a previous study of pesticide toxicity to CAR cells showed that pH values can indicate significant metabolic alterations at subcytopathic levels of pesticide (Shea and Berry 1982a).

Examination of carbaryl-treated cultures for interferon activity,

Table 1: Effect of carbaryl (1ppm) on CAR cultures

	carbaryl	control
# cells ($x10^{-5}$)/culture	2.7	2.4
protein (mcg)/culture	283	275
cytotoxicity	-	-
pH of medium	7.0	7.05
% adherence upon passage	83	80

Table 2: Decreased interferon in carbaryl-treated cultures as measured by destruction of cells by viral infection

		Medium added to secondary cultures	
carbaryl	stock medium	uninfected culture supernatant	infected culture supernatant
+	10*	10	18
-	10	10	100

*Values represent percentage of dye bound by cultures, with the amount bound by cultures not treated with carbaryl and receiving infected culture supernatant defined as 100%.

however, revealed significant differences from untreated cultures. Using the dye-binding assay of Armstrong (1971), supernatants from infected cultures not treated with carbaryl were found to provide 10 times as much antiviral protection as supernatants from uninfected cultures with or without carbaryl pretreatment. In contrast, carbaryl-treated cultures provided only twice as much antiviral protection as uninfected cultures (Table 2). The extent of dye-binding by cultures receiving supernatants from uninfected cultures was identical to the baseline levels of cultures receiving stock medium. Carbaryl treatment alone did not alter the levels of dye bound in the presence of either stock medium or supernatants from uninfected cultures (Table 2).

A similar relationship was observed when assaying the amount of infectious viral progeny synthesized in the presence of the various supernatants (Table 3). Virtually identical levels of virus were synthesized with either stock medium or supernatants from

Table 3: Decreased interferon in carbaryl-treated cultures as measured by viral progeny synthesis

	Medium added to secondary cultures		
carbaryl	stock medium	uninfected culture supernatant	infected culture supernatant
+	100*	98	66.4
-	100	94	9.7

*Values indicate percentage of viral progeny synthesized, with the amount synthesized in cultures not treated with carbaryl and receiving stock medium defined as 100%.

uninfected cultures, both with and without carbaryl pretreatment. Only 10% of the amount synthesized in stock medium was synthesized in the presence of the supernatants from infected cultures not pretreated with carbaryl. Far less antiviral protection was provided by the supernatants of infected cultures which were pretreated with carbaryl as these secondary CAR cultures synthesized over 60% of the amount of virus synthesized in control cultures (Table 3).

It is proposed that 1ppm carbaryl imposes a mild inhibition of (certain aspects of?) cellular metabolism, clearly undetectable by the parameters of cytotoxicity employed by this study, and that this mild suppression is rendered functionally acute with respect to interferon biosynthesis by subsequent viral infection. The replication of GFV-2 is enhanced in vitro by carbaryl (Shea 1983a) and by its hydrolysis product 1-naphthol (Shea 1983b), and the observed inhibition of interferon synthesis by carbaryl suggests a possible mechanism for this enhancement (Shea and Berry 1984). A similar correlation was demonstrated between suppression of interferon synthesis and enhancement of encephalomyocarditiitis virus in L-929 cells by the emulsifier Toximul (Lee et al. 1980).

Dickson (1982) stresses that an inherent caveat in acute toxicity tests of acquatic organisms under laboratory conditions is that general population dynamics may profoundly influence environmental toxicity levels, rendering extrapolation from laboratory data speculative.

In addition to considerations of favorable versus adverse environmental conditions, these results suggest that the superimposition of a viral infection in a population exposed to pesticides may alter the effective toxicity of the compound. As viral infections are certainly common, the possibility of such synergistic amplifications of toxicity should be considered prior to prediction of environmentally "safe" levels.

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