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Chronic Exposure of Goldfish-derived Cell Cultures to Toxaphene Alters the Replication of Goldfish Virus-2

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In a recent examination of the effect of toxaphene on the goldfish-derived CAR cell line, it was demonstrated that cells chronically exposed to low levels of toxaphene were more sensitive to subsequent exposure to increased concentrations of this insecticide than were cells which had not been previously exposed (SHEA & BERRY 1982).

To further explore this phenomenon, we examined the response of chronically exposed cells to infection by Goldfish Virus-2.

MATERIALS & METHODS

Cells and virus: cultivation of the goldfish-derived CAR cell line (ATCC CCL-71) has been previously described (SHEA & BERRY 1982). Goldfish Virus-2 (GFV-2), an Iridovirus, was originally isolated from air bladder cultures of healthy goldfish in this laboratory (BERRY & SHEA 1982); viral growth studies have indicated GFV-2 replication is completed by 5 days in CAR cell cultures at 25°C.

Medium: Medium 199 was employed, supplemented with 10% fetal bovine serum, and 100 units of penicillin and streptomycin and 0.025 mcg. Fungizone/mL (Gibco).

Toxaphene: technical grade toxaphene (99.5% pure) was obtained from Hercules Corp., Delaware. Stock solutions were prepared in 100% ethanol at 0.2g/mL and serially diluted with medium to final concentrations of 50, 35, 25, 15, 10, and 5 parts per million (ppm).

Chronic exposure of cells: cells were chronically exposed to toxaphene at 5ppm for 100 days as previously described (SHEA & BERRY 1982). In brief, cells were planted in 150cm^2 flasks (Corning) at a density of 2x10^6/flask in 20mL medium without toxaphene and incubated for 24hr at 25°C. Medium was then replaced with 20mL medium containing 5ppm toxaphene. At 10-day intervals, cells were subcultured, with the above proceedure repeated.

Infection of chronically-exposed cells: after 100 days chronic exposure to 5ppm toxaphene (above), cells were planted in 24-well trays (Falcon) at a density of 1x10^5/well in medium without toxaphene; these cells were termed CAR_5. Control cells (CAR_0) were passaged in identical manner in medium without toxaphene. 24hr later, medium was removed, and duplicate cultures received 2x10^{6}TCID50 GFV-2. After allowing 2hr for viral adsorption, cultures were rinsed and refed with medium without toxaphene. The viral inoculum was retained for titration of unadsorbed virus. Viral progeny were harvested on day 5 after infection. TCID50 were determined by endpoint dilution using the microtitration system (BERRY & SHEA 1982). Total protein/culture was determined according to OYAMA & EAGLE (1956).

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RESULTS & DISCUSSION

Cells chronically exposed to 5ppm toxaphene for 100 days exhibit mild granulation but otherwise appear normal, and replicate at a rate equivalent to that of cells not exposed to toxaphene (SHEA & BERRY 1982).

Chronically-exposed cells (CAR_5) and cells not treated with toxaphene (CAR_0) were planted at equivalent densities (Table 1) and infected with GFV-2. Both the time course and extent of viral-induced cytopathology was identical for CAR_5 and CAR_0 as monitored by phase-contrast microscopy, but examination of viral progeny showed nearly 10-fold fewer progeny produced in CAR_5 than in CAR_0. The possibility existed that uninfected CAR_0 cells continued to grow at a rate beyond that of CAR_5 during the 5-day incubation. If this were the case, it could conceivably account for increased viral progeny synthesis. However, determination of the total protein/infected culture discounted this possibility, as there was no significant difference in protein/culture; the amount of viral progeny produced/ug protein in infected cultures (Table 1) for CAR_5 versus CAR_0 showed nearly a 10-fold difference.

Table 1: Effect of chronic exposure of CAR cells to toxaphene on GFV-2 replication

# cells x 10^5/well at time of infection	
CAR_0	CAR_5
3.9	5.4
2.2 (3.1)*	4.4(4.9)
Unadsorbed viral inoculum 2hr after infection(TCID50/mL)	
CAR_0	CAR_5
$4.0x10^5$	$8.6x10^5$
$4.0x10^5$ ($4.0x10^5$)	$1.3x10^6$ ($1.1x10^6$)
Viral progeny day 5 after infection(TCID50/mL)	
CAR_0	CAR_5
$4.0x10^6$	$4.0x10^5$
$4.0x10^6$ ($4.0x10^6$)	$8.6x10^5$ ($6.3x10^5$)
Total protein day 5 after infection(ug/infected culture)	
CAR_0	CAR_5
140	110
95 (117.5)	145 (127.5)
(TCID50/culture)/(ug protein/culture)	
CAR_0	CAR_5
$3.4x10^4$	$4.9x10^3$
Progeny TCID50 produced/TCID50 adsorbed from inoculum	
CAR_0	CAR_5
12.5	3.5

*duplicate determinations shown; averages in parentheses

CAR_5 adsorbed significantly less virus than CAR_0 (Table 1); it at first seems that this is the reason for synthesis of fewer progeny

in CAR_5 than in CAR_0. However, determination of the progeny virus produced/adsorbed virus demonstrates a ratio of 12.5 for CAR_0, yet only 3.5 for CAR_5. Approximately 3.6 times as much infectious progeny was produced per adsorbed virus by CAR_0 as was produced by CAR_5. Thus, although adsorption of GFV-2 is restricted by chronic exposure of CAR cells to toxaphene, this alone cannot account for the observed restriction in progeny synthesis.

A previous study (SHEA & BERRY 1982) has shown that toxaphene is taken up by CAR cells, and localizes in the alcohol-soluble cellular fraction; indications of metabolic interactions between pesticide and cells were also observed. It was further demonstrated that chronic exposure of CAR cells to low levels of toxaphene resulted in increased sensitivity to subsequent exposure to increased concentrations. The present study has demonstrated that chronically passaged cells are restricted in an undisclosed manner in both the adsorption and replication of Goldfish Virus-2.

These results represent a situation differing from that of an earlier study, in which chronic exposure of HeLa cells to several insecticides enhanced poliovirus replication (GABLIKS 1965).

Goldfish have been shown to concentrate toxaphene in their fatty tissue 200-fold over that of the environment (HUNT & KEITH 1960) Since toxaphene has until recently been the most widely used organochlorine insecticide in the United States (CASIDA et al. 1974) and is still in use in other countries, and has been shown to persist in the environment for as long as 10 years (EPA TOXAPHENE STATUS REPORT 1971) further studies on the nature of the interaction of this pesticide with piscine cells are warranted.

REFERENCES

BERRY,E.S. and T.B.SHEA: Abstracts 82nd Amer. Soc. Micro. S77, 247(198

CASIDA,J.E., R.L.HOLMSTEAD, S.KHALIFA, J.R.KNOX, T.OHSAWA, K.J.PALMER and R.Y.WONG: Science 183, 520 (1974)

GABLIKS,J.: Soc. Exp. Biol. Med. 120, 172-175 (1965)

HUNT,E.G. and J.O.KEITH: Proc. 2nd. Conf. Use Agricult. Chem. Calif. 1 (1963)

OYAMA,V.I. and H.EAGLE: Proc. Soc. Exp. Biol. Med. 91, 305 (1956)

SHEA,T.B. and E.S.BERRY: Bull. Environ. Contam. Toxicol. 29,68-75 (198

U.S. ENVIRONMENTAL PROTECTION AGENCY: Toxaphene Status Report. Office of Pesticide Programs. Wash., D.C. (1971)

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