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MAMMALIAN TOXICITY OF AN INSECT IRIDOVIRUS

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Summary. — Intraperitoneal injection of large doses of untreated and UV-inactivated *Chilo* iridescent virus (CIV) an insect iridovirus caused acute death of mice, while the heat-inactivated virus had not any toxic effect. The LD_{50} value of untreated CIV for mouse was 206 μg (approximately 9.6 mg/kg body weight). Antiserum against CIV specifically neutralized the lethal toxicity of CIV in mice.

Key words: *Chilo iridescent virus*; *insect iridovirus*; *mouse toxicity*

Introduction

Icosahedral cytoplasmic deoxyriboviruses with iridescent property have been isolated from the two classes of Arthropoda, Insecta and Crustacea (Kelly and Robertson, 1973; Federici and Hazard, 1975; Federici, 1980; Cole and Morris, 1980). This virus group is currently classified as the genus *Iridovirus* of the family Iridoviridae (Matthews, 1979). Usually, arthropod iridoviruses have wide host ranges (Smith *et al.*, 1961; Day and Dudzinski, 1966; Hama, 1968; Ohba, 1975*b*) and some viruses are capable of replicating in two or three classes of Arthropoda (Ohba and Aizawa, 1979*a*; Cole and Morris, 1980; Ohba *et al.*, 1982). McIntosh and Kimura (1974) demonstrated in vitro multiplication of *Chilo* iridescent virus (CIV), an insect iridovirus isolated from the rice stem borer, *Chilo suppressalis* (Fukaya and Nasu, 1966), in a poikilothermic vertebrate cell line. However, no in vivo replication of arthropod iridovirus has been shown in vertebrates (Kelly and Robertson, 1973).

In our studies on the responses of poikilothermic vertebrates to arthropod iridoviruses, intraperitoneal injection of large doses of arthropod iridoviruses showed lethal toxicity to the frog, *Rana limnocharis*, without virus multiplication (Ohba and Aizawa, 1981). In this paper, we report a mammalian toxicity of CIV, which causes acute death of the mouse.

Materials and Methods

CIV was propagated in the larvae of the greater wax moth, *Galleria mellonella*. Infected larvae and pupae were collected 10-14 days postinjection and triturated in 0.05 M phosphate buffer, pH 7.2. Virus was partially purified by differential centrifugation at 7.000 rev/min for 10 min and at 12,000 rev/min for 30 min. Further purification was performed by chloroform treatment and

Table 1. Toxicity of Chilo iridescent virus in mice

Virus preparation	Hours post injection									Total
	< 18	21	22	23	27	32	34	37	240	
Untreated	0	0	1*	0	1	1	1	1	—	5/5**
UV-inactivated	3	1	0	1	—	—	—	—	—	5/5
Heat-inactivated	0	0	0	0	0	0	0	0	0	0/5
Control***	0	0	0	0	0	0	0	0	0	0/5

* Number of dead mice.
** Number of dead versus tested.
*** 0.05 M phosphate buffer, pH 7.2.

two cycles of Percoll (Farmacia Fine Chemicals, Sweden) density gradient centrifugation as previously described (Ohba nad Aizawa, 1979*b*). Concentrated purified virus suspension was finally prepared in 0.05 M phosphate buffer, pH 7.2. Virus concentration (μg protein/ml) was determined according to the method of Lowry *et al.* (1951) by using bovine serum albumin as standard.

Inactivation of the virus infectivity was performed as previously described (Ohba and Aizawa, 1979*b*). Purified virus suspension (0.5 ml) was placed in a Petri dish (6 cm in diameter) and irradiated with UV light for 40 min at a distance of 15 cm from a Toshiba germicide lamp (15 W). Another virus sample was inactivated by heating at 60 °C for 20 min.

Antisera against CIV, *Tipula* iridescent virus (TIV) (Xeros, 1954), and *Armadillidium* iridescent virus (AvIV) isolated from a terrestrial crustacean (Federici, 1980) were prepared according to the method previously described (Ohba and Aizawa, 1981) and heated at 56 °C for 30 min prior to use. Infectivity neutralization titer of antiserum was assayed by a dilution endopoint technique using *Galleria* larvae (Ohba and Aizawa, 1979*b*) and calculated according to Reed and Muench (1938). Neutralization titres of antisera for CIV, TIV, and AvIV were 1 : 1024, 1 : 202, and 1 : 256, respectively.

Male mice of the strain ddY (body weight: 20—23 g) were injected intraperitoneally with 0.5 ml of virus suspension containing 4.6 mg virus protein and reared for 10 days. Penicillin (100 units/ml) and streptomycin (100 μg/ml) were added into virus suspension prior to injection. The dose response was estimated by inoculation of the virus suspension diluted 1 : 2, 1 : 10, 1 : 50 and 1 : : 250; the LD_{50} value (μg virus protein per mouse) was calculated according to Reed and Muench (1938). To test the effect of antisera on the toxicity of CIV to mice, purified virus suspension containing 10 LD_{50}/0.5 ml was mixed with an equal volume of undiluted antiserum. After incubation at 27 °C for 1 hr, 0.5 ml of the mixture was injected intraperitoneally into mice. Normal rabbit serum was used as control.

Results and Discussion

As shown in Table 1, intraperitoneal injection of large doses of untreated (infective) and UV-inactivated CIV preparations was lethal to mice, while there was no death in mice injected with heat-inactivated virus during the test period of 10 days. Mice injected with untreated and UV-inactivated viruses showed clinical signs of lowered activity. In contrast, the activity of mice injected with heat-inactivated CIV or 0.05 M phosphate buffer, pH 7.2, was normal. Since the lethality was demonstrated within 37 hr by untreated and within 23 hr by UV-inactivated CIV and the critical temperature for CIV multiplication is 35 °C (Fukaya, 1968; Ohba, 1975*a*), it is unlikely that the death of mice was caused by virus multiplication.

Table 2. Dose response of toxicity of Chilo iridescent virus in mice

Virus dose	No. of dead/No. of tested
2.300*	5/5
460	5/5
92	0/5
18.4	0/5
Control**	0/5

* μg protein per mouse.
** 0.05 M phosphate buffer, pH 7.2.

Dose response study was performed using untreated CIV. The LD_{50} value was 206 μg virus protein per mouse, approximately 9.6 mg/kg body weight (Table 2). This value is comparable to that (ca. 10.6 mg/kg body weight) of AvIV for the frog, *R. limnocharis* (Ohba and Aizawa, 1981).

The effect of antisera on the mouse toxicity of CIV is shown in Table 3. All of 5 mice injected with CIV treated with the homologous antiserum survived during the test period of 10 days. In contrast, antisera against TIV and AvIV and normal rabbit serum did not inhibit the toxicity of CIV to mice.

The nature of the lethal factor in the present study is very similar to those of the toxic factor destructive for *Galleria* larval hemocytes (Ohba and Aizawa, 1979*b*), the in vitro cytopathic factor (Cerutti and Devauchelle, 1979; M. Ohba, K. Kanda, and K. Aizawa, in preparation), and the frog-killing factor (Ohba and Aizawa, 1981) present in virions of CIV and AvIV. These results suggest that the toxicities demonstrated in the different combination of viruses and hosts are caused by the same proteinous factor.

Kirn *et al.* (1972) reported that intraperitoneal inoculation of a large dose of the frog virus 3 (FV 3), a vertebrate iridovirus isolated from *Rana pipiens* (Granoff *et al.*, 1966), caused an acute death of mice without virus multiplication. Since this effect of FV 3 was not inhibited by γ-irradiation nor by heat treatment the nature of the toxic factor of CIV is different from that of FV 3.

The target organ for the toxic factor of CIV is still uncertain. However, the liver is suspected as the target organ in a pathological study on the mouse toxicity of CIV (S. Belloncik, personal communication).

Table 3. Effect of antisera on the toxicity of Chilo iridescent virus in mice

Treatment of virus	Lethality
CIV antiserum	0/5*
TIV antiserum	5/5
AvIV antiserum	5/5
Normal rabbit serum	5/5

* Number of dead versus tested.

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