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In vivo antiviral effect of 9-(2-hydroxymethyl) guanine on experimental infection of chum salmon (*Oncorhynchus keta*) fry with *Oncorhynchus masou virus* (OMV)

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The therapeutic efficacy of 9-(2-hydroxyethoxymethyl) guanine (Acyclovir, ACV) was evaluated using *Oncorhynchus masou virus* (OMV) and chum salmon fry. The fish, which were experimentally infected with OMV, were treated with ACV either orally or by the immersion method. Daily immersion of fish into ACV solution (25 μg/ml, 30 min/day, 15 times) reduced mortality of the infected fish. Oral administration of the drug (25 μg/fish per day, 60 times) did not affect survival of the chum salmon. On the contrary, the group administered 5-iodo-2'-deoxyuridine (IUdR) by the oral route showed a higher survival than the ACV-administered group. This suggested that an effective level of ACV was not maintained in fish given the drug by the oral route.

Daily immersion of infected fish into ACV solution (25 μg/ml, 30 min/day, 60 times) considerably suppressed the development of tumors induced by OMV.

Acyclovir; *Oncorphynchus masou virus*; chum salmon; survival tumor suppression

Introduction

Acyclovir [9-(2-hydroxyethoxymethyl) guanine, ACV] has been shown to have anti-herpesvirus activity against mammalian virus infections [1,2,4,5,10,11,13]. The effect of ACV is restricted to herpesviruses. Once introduced into cells, ACV is converted to the monophosphate form by viral thymidine kinase. It is then converted to the triphosphate form and acts by inhibition of the viral DNA polymerase [3].

Oncorhynchus masou virus (OMV) is a virulent herpesvirus of salmonids and induces tumors in infected fish that survive [6–9]. In the present study, we evaluated the therapeutic efficacy of ACV on OMV infection in vivo.

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Materials and methods

Virus and chemicals

An isolate of OMV from cultured masou salmon (*Oncorhynchus masou*) was passed in RTG-2 cells and stored at -80°C until used. The ACV (sodium salt), supplied by the Wellcome Foundation Ltd., London, U.K., was dissolved in water at a concentration of 1000 μg/ml by warming at 30°C. The 5-iodo-2′-deoxyuridine (IUdR, Wako Pure Chemical Co. Ltd., Tokyo, Japan) used as a reference drug was prepared by the same way.

Experimental fish

Two lots of chum salmon (*Oncorhynchus keta*) fry, body wt 0.44 and 0.69 g, used in this study were distributed in groups of 50 each and held in 10–18°C running water.

Experimental infection

The fish were exposed to the virus by placing them in 15°C water containing approximately 100 $TCID_{50}$/ml of OMV for 1 h before water flow was resumed. Control groups were treated by the same method with cell culture medium instead of virus.

Drug treatment

Immersion method

The fish were immersed for 30 min in a solution containing 25 μg/ml of ACV. Normal controls and virus controls underwent the same handling stress without the drug. This operation was performed before and 30 min after virus infection, and then at 1-day intervals during the appropriate period (see Fig. 1 and Table 1).

Oral administration

The fish in the experimental group were fed an artificial diet (Nihon Haigo Shiryo Co., Tokyo, Japan, No. 3c) containing 25 μg of ACV/fish per day. Feedings of the diet containing the drug were continued during 60 days. Included as controls were a group fed IUdR 25 μg/fish per day, a group fed ACV but without virus infection and a group exposed to virus but without drug administration.

Results

Effect of ACV on survival of OMV-infected fish

Severe mortality in the virus control group began 12 days after virus infection, and 90% of the fish succumbed in the ensuing 56 days (Fig. 1). On the other hand, the sur-

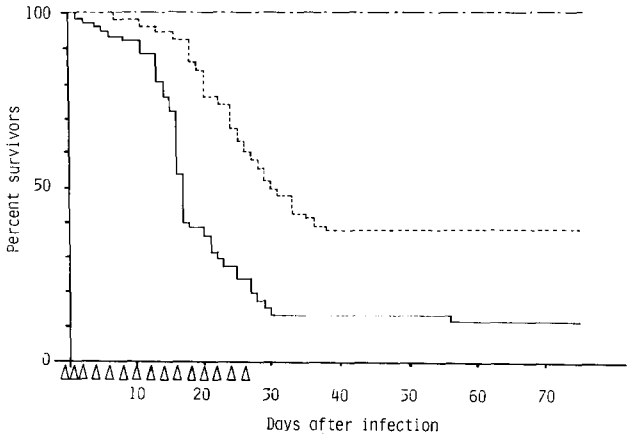

Fig. 1. Effect of ACV treatment by immersion on the survival of 5-mth-old chum salmon fingerlings (body wt = 0.44 g) following infection with OMV. OMV inoculation = 100 $TCID_{50}$/ml. ——— = virus control (no drug treatment); —·—·— = negative control (neither virus infection nor drug treatment); ------- = drug treatment group (ACV immersion in 25 μg/ml, 30 min daily intervals, 15 days); △ = drug immersion.

vival rate of the drug immersion group was 30% higher than that of the controls. The mortality in the drug treated group was relatively delayed. No death caused by handling stress was observed.

When ACV was given orally, the mortality in the treated group was delayed slightly. However, by the end of the experiment there was no difference in mortality between the control and experimental groups. Oral administration of IUdR was more effective than ACV (Fig. 2).

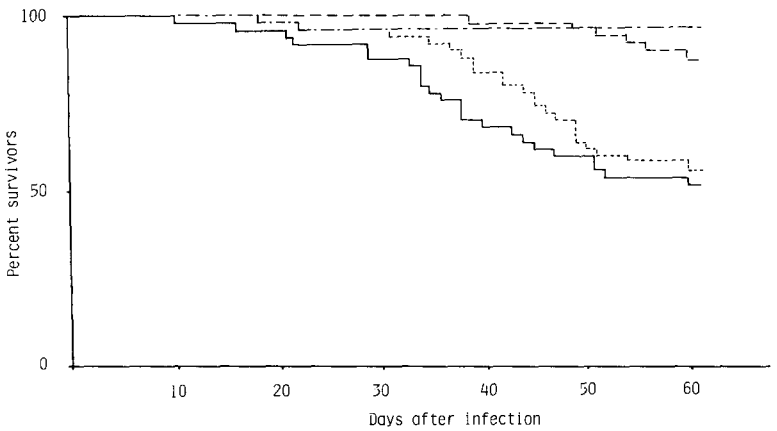

Fig. 2. Effect of oral administration of ACV and IUdR on the survival of 4-mth-old chum salmon fingerlings (body wt 0.69 g) following infection with OMV. OMV inoculation = 100 $TCID_{50}$/ml. ——— = virus control (no drug treatment); —·—·— = drug control (no virus infection); ------- = ACV administered orally (25 μg/fish per day for 60 days); ——·—·— = IUdR administered orally (25 μg/fish per day for 60 days).

TABLE 1

Effect of ACV treatment on the development of tumors in OMV (*Oncorhynchus masou virus*) infected chum salmon

Group	Treatment period	Tumor development[a]
Virus control	V.I.[b] 60 120	11/24
ACV treatment[c]	V.I. 60 120	0/34
ACV treatment[c]	V.I. 15 60 120	15/27
ACV treatment	V.I. 15 60 120	1/29
IUdR treatment[d]	V.I. 60 120	3/27
Drug control (ACV)	60 120	0/26
Drug control (IUdR)	60 120	0/37

[a] Number showing tumors/total number.
[b] Virus infection by immersion.
[c] Daily immersion in containing 25 μg/ml ACV.
[d] Daily immersion in containing 25 μg/ml IUdR.

Effect of ACV on tumor induction by OMV

Tumor induction was observed in surviving fish 120 days after virus infection. The body surface, oral cavity, gills and internal organs of fish were examined 120 days post-infection. No tumor induction was observed in the drug control groups. The incidence of tumors in experimental fish is shown in Table 1. In the virus control infected with OMV but not treated with the drug, tumor induction was observed in 11 of 24 fish (46%). In those fish treated 60 times with ACV, no tumors were observed. In the IUdR group (treated using the same schedule as ACV), tumors were observed in 3 of 37 fish (8%). In a group treated 15 times with ACV following virus infection, no suppressive effect of the drug on tumor induction was observed (15/27, 56%). However, the suppressive effect of the drug was observed in the group which was treated 45 times by ACV from day 15 to day 60 after virus infection (1/29, 3.4%). In all cases, tumor development was observed around the mouth, but not in other locations.

Discussion

A study by Schaeffer et al. [12] indicates that ACV had a prophylactic effect without toxicity against HSV-1 infection in mice and rabbits. Several investigators have

reported the anti-herpesvirus effect of ACV in animal models such as mice [4,5,10,11] or rabbits [1,13]. In this study, the authors used OMV and chum salmon to look for enhanced survival and tumor suppression in vivo.

The immersion method (25 μg/ml, 30 min/day, 15 times) and oral administration (25 μg/fish per day) were adopted in the survival study in order to deliver the drug to fish. In the case of immersion, ACV was shown to improve the survival of the OMV infected fish. This result suggests the adsorption of the ACV through the gills or body surface. Administration of ACV by the oral route did not prevent the death of infected fish. However, IUdR showed efficacy when administered by the same method. This observation suggests that an effective level of ACV was not maintained in the fish when given by the oral route. It seems that the difference in the efficacy of each method of administration might be due to the adsorption of ACV by the fish, and its distribution and metabolic disposition in the fish. In further studies, drug levels in fish tissues should be examined.

With the immersion method, considerable suppression of tumor induction was observed in the group treated with ACV 60 times following OMV infection and in the group treated 45 times during day 15 to day 60 after virus infection. Results obtained in this study suggest that ACV could be effective in preventing herpesvirus-induced tumors. Further study will be necessary to clarify the mechanism of action of ACV on tumor induction.

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