Antiviral Research, 6 (1986) 233–239
Elsevier

AVR 00204

Antiviral effect of two aryl-oligopeptides, FR41565 and FR48217

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(Received 9 July 1985; accepted 7 October 1985)

Summary

Macrophages from mice pretreated with two chemically synthesized immunostimulating aryl-oligopeptides, FR41565 and FR48217, inhibited the multiplication of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in monkey Vero cells, and that of vesicular stomatitis virus (VSV) in murine L929 cells. In addition, the aryl-oligopeptides protected mice against a lethal HSV-1 infection. In particular, when treated with FR48217 at 6 mg/kg, all mice survived, whereas all control mice died from the HSV-1 infection.

herpes simplex virus; oligopeptides; interferons; macrophages

Introduction

Macrophages play an important role in host resistance to viral infections, bacterial infections and tumor growth [10]. Focal infiltration of mononuclear cells is the first cellular response toward infection with herpes simplex virus (HSV) in mice [4].

Also, macrophages have been reported to suppress the multiplication of viruses such as HSV and cytomegalovirus in cell culture, and contact of macrophages with the virus-infected cells is required for the manifestation of this antiviral activity [10,12,13].

Macrophage inhibitors are known to decrease resistance to HSV infection [7,15], while macrophage activators increase resistance to this virus [6,11]. FK156, a peptide isolated from culture fluids of *Streptomyces* [1], has been shown to enhance host defenses against microbial infections, such as *Escherichia coli* and *Pseudomonas aerugi-*

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nosa, and to protect mice from death following such infections [9]. This agent increases the number and the activity of macrophages [8].

In the present study, the antiviral activity of two other aryl-oligopeptides, analogues of FK156, was investigated.

Materials and Methods

Compounds

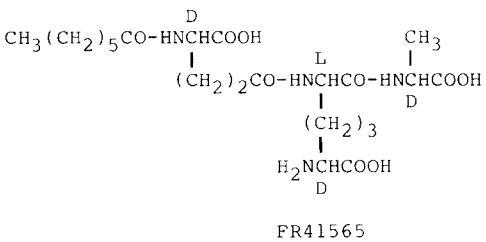
Two aryl-oligopeptides (FR41565 and FR48217), synthesized by methods previously described [3], were kindly provided by Fujisawa Pharmaceutical Co., Osaka, Japan (Fig. 1). The oligopeptides were dissolved in 0.9% saline. The LD_{50} values of these compounds for mice were greater than 500 mg/kg.

Mice

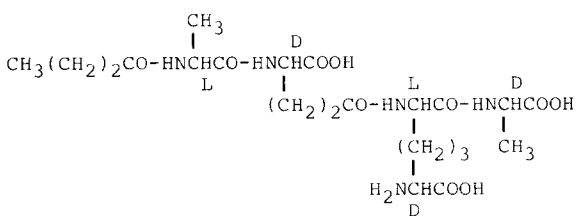
4-week-old female ICR mice were purchased from Clea Japan, Osaka, Japan. Each experimental group consisted of 5 mice, and the control group consisted of 6 mice.

Cells and viruses

L929 cells, FL cells and Vero cells were grown in Eagle's minimal essential medium (EMEM) supplemented with 10% newborn calf serum (NCS; Irvine Scientific, Santa Ana, CA). Vesicular stomatitis virus (VSV, New Jersey strain), herpes simplex virus



FR41565



FR48217

Fig. 1. Chemical structures of FR41565 and FR48217.

type 1 (HSV-1, Miyama strain) and type 2 (HSV-2, UW238 strain) were used. VSV was propagated in FL cells, and HSV-1 and HSV-2, in Vero cells. The titers of VSV, HSV-1 and HSV-2 were determined by a plaque assay technique. Briefly, monolayer cell cultures in 24-well microplates (Nunc, Inter Med, Denmark) were inoculated with serially diluted virus suspensions (0.1 ml/well) and incubated for 1 h at 37°C in a humidified 5% CO_2 atmosphere to allow virus adsorption. After removal of unadsorbed virus, EMEM containing 1% NCS and 0.5% methylcellulose (Muromachi Chemicals Co., Tokyo, Japan) was added, and the cells were fixed and stained with Wright's solution 2 or 3 days later.

Preparation of peritoneal exudate cells

Mice were treated with FR41565 or FR48217 subcutaneously either once at a dose of 6 mg/kg or daily for 4 days at a dose of 0.2 mg/kg per day. Four days after the first injection, peritoneal lavage was done with 5 ml phosphate-buffered saline containing 10 IU/ml of heparin. Peritoneal exudate cells (PEC) were washed twice with serum-free EMEM and suspended in EMEM supplemented with NCS. Approximately 60–70% of the PEC adhered to plastic and resembled macrophages morphologically.

PEC obtained from untreated mice were used as controls.

Assay of antiviral activity

Antiviral activity was assayed according to the method of Morahan et al. [10]. Briefly, confluent L929 cells and Vero cells in 24-well microplates were infected with 20 plaque-forming units (PFU) of VSV and HSV, respectively. After virus adsorption for 1 h at 37°C, 2×10^5 PEC were added to the L929 or Vero cell cultures and allowed to adhere to them for 2 h at 37°C in a humidified 5% CO_2 atmosphere. Non-adherent cells were removed by washing with EMEM. EMEM containing 10% NCS was added, and the plates were incubated for 2–3 days. After the cells had been frozen once and thawed, the culture supernatants were collected and stored at -80°C before virus titration.

Infection in mice

Mice were inoculated intraperitoneally with 30 LD_{50} of HSV-1; 1 h later, the oligopeptides were administered subcutaneously daily for 4 days at a dose of 0.2 mg/kg during 3 weeks, or once a week at a dose of 6 mg/kg during 3 weeks.

Effect of FR41565 and FR48217 on interferon induction by poly (I:C)

FR41565 and FR48217 were administered subcutaneously at a dose of 6 mg/kg, 0, 24 or 72 h before intraperitoneal injection with poly (I : C) (100 μg). Blood was drawn 1, 3 and 6 h after poly (I : C) injection. Interferon activity in the serum was assayed by the dye uptake method described previously [2].

Results

Inhibition of virus multiplication by PEC

Mice were injected with FR41565 or FR48217 at a dose of 6 or 0.2 mg/kg, and the antiproliferative activity of PEC from the mice treated with the compounds was examined in cells infected with VSV, HSV-1 or HSV-2. Table 1 shows that the PEC obtained from the mice treated with the compounds significantly suppressed multiplication of VSV, HSV-1 and HSV-2. Significant inhibitory effects were noted, for the VSV-infected cells, with FR41565 at 0.2 mg/kg ($P<0.0005$) and FR48217 at 6 or 0.2 mg/kg ($P<0.0005$ and $P<0.01$, respectively); for the HSV-1-infected cells, with FR41565 or FR48217 at 0.2 mg/kg and 6 mg/kg ($P<0.0001$). In particular, PEC from mice treated with FR48217 completely suppressed the multiplication of HSV-1 in Vero cells. Also, HSV-2 replication was inhibited to a significant extent with FR48217 ($P<0.0001$–0.01). Control PEC from the non-treated mice inhibited the multiplication of VSV in L929 cells, but not that of HSV in Vero cells.

Protective effect of aryl-oligopeptides against HSV infection in mice

All control (untreated) mice died within 6 days after virus infection. Treatment with

TABLE 1

Virus titers in the supernatants of HSV- or VSV-infected-cell cultures mixed with macrophages from mice treated with FR41565 or FR48217

Virus	Macrophages from mice treated with					Virus control[b]
	None[a]	FR41565		FR48217		
		6 mg/kg	0.2 mg/kg	6 mg/kg	0.2 mg/kg	
HSV-1	4.36 ±0.15	0.56* ±0.72	0.41* ±0.64	not detectable*	not detectable*	4.63 ±0.23
HSV-2	3.08 ±0.32	1.82**** ±0.63	1.51*** ±0.46	0.80* ±0.34	1.12* ±0.38	3.18 ±0.18
VSV	4.43 ±0.17	4.39 ±0.45	3.07** ±0.39	3.08** ±0.37	3.39**** ±0.43	6.72 ±0.07

Macrophages were prepared from mice treated with or without FR41565 or FR48217 at a dose of 6 or 0.2 mg/kg. They were added to L929 cells infected with vesicular stomatitis virus (VSV), and to Vero cells infected with herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) 1 h after inoculation of virus at a density of 2×10^5 cells/well. The supernatants were collected 2 to 3 days later. Virus titers in the supernatants were assayed by the plaque assay method and expressed as the logarithm of mean ± S.D. (PFU) in quadruplicate determinations. *, **, *** and **** indicate that there is a significant difference in virus titers between the cell cultures incubated with macrophages from drug-treated mice and the cell cultures incubated with macrophages from non-treated mice[a] or without macrophages[b], by using Student's *t*-test. * $P<0.0001$; ** $P<0.0005$; *** $P<0.001$; **** $P<0.01$.

[a] "None" indicates virus titers in the supernatant of cell cultures mixed with macrophages from untreated mice.

[b] Virus control refers to virus titers in the supernatant of cell cultures incubated in the absence of macrophages.

either oligopeptide prolonged the survival time, or completely protected the mice from the lethal HSV infection (Table 2). There were significant differences in mortality and survival time between the group treated with FR48217 at 6 mg/kg and the non-treated control group ($P<0.001$), between the group treated with FR48217 at 0.2 mg/kg and the control group ($P<0.05$), and between the group treated with FR41565 at 0.2 mg/kg and the control group ($P<0.05$). All mice treated with FR48217 at 6 mg/kg were still alive 70 days after the inoculation of HSV-1.

Effect of FR41565 and FR48217 on interferon induction by poly (I:C)

When poly (I:C) was injected to the mice, higher interferon activities were detected in the sera of the mice treated with FR41565 and FR48217 than in the sera of the mice which were not treated with the aryl-oligopeptides (Table 3). Thus, FR41565 and FR48217 enhanced the interferon response to poly (I:C) in mice.

TABLE 2

Antiviral effect of two aryl-oligopeptides in mice infected with herpes simplex virus (HSV)

		Number of mice alive at day							Survival rate
		4	5	6	7	8	9	10	
Control		6	3	0	0	0	0	0	0/6
FR41565	6 mg/kg	5	5	2	2	2	1	1	1/5
	0.2 mg/kg	5	5	5	3	2	2	2	2/5
FR48217	6 mg/kg	5	5	5	5	5	5	5	5/5
	0.2 mg/kg	5	5	5	2	2	2	2	2/5

TABLE 3

Effect of FR41565 and FR48217 on the interferon induction by poly (I:C) in mice

Time of administration of compounds (h)	Oligo-peptides	Interferon activity in the serum (IU/ml) (hours after the administration of poly (I:C))		
		1	3	6
0	FR41565	90	4597	<22
	FR48217	<22	4597	713
-24	FR41565	32	4254	357
	FR48217	n.d.	2010	505
-72	FR41565	<22	175	357
	FR48217	<22	505	357
Control		<22	713	<22

n.d., not determined.

Discussion

Two aryl-oligopeptides, FR41565 and FR48217, have been reported to augment the number and activity of macrophages [3]. In the present study, peritoneal exudate cells (PEC) harvested from mice treated with FR41565 or FR48217, reduced VSV and HSV multiplication in cell culture. PEC from mice treated with FR48217 at 0.2 mg/kg or 6 mg/kg completely inhibited the multiplication of HSV-1.

Although the mechanism by which aryl-oligopeptides suppress the multiplication of viruses is not yet clear, the following factors may be considered: (i) activation of the macrophages; (ii) induction of interferon in PEC upon activation by the oligopeptides; (iii) enhancement of interferon production by the oligopeptides; (iv) enhancement of NK cell activity. Evidence that their factors are involved in the mechanism of action of the aryl-oligopeptides is as follows.

PEC from mice treated with *Propionibacterium parvum* suppress the multiplication of HSV, and the reticuloendothelial system plays an important role in the host defense against HSV infection [14].

Secondly, FR41565 and FR48217 bring about a 6-fold increase in interferon production in mice stimulated with poly (I : C) either 0 or 24 h after previous administration of FR41565 or FR48217. Yet, the latter compounds are not potent interferon inducers per se in ICR mice. Also, the protection of FR41565 and FR48217 against HSV infection in vivo may be mediated at least in part by an increased interferon response to HSV.

Thirdly, when the cytotoxicity to YAC-1 cells of NK cells of mice treated with FR41565 was determined by the usual radioactive chromium release assay method, NK activity was clearly enhanced by FR41565 treatment [5].

Thus, the antiviral activity of the aryl-oligopeptides may be related to direct inhibition of virus multiplication by activated macrophages, augmentation of NK activity and/or enhanced interferon production.

All mice infected with HSV-1 survived following treatment with FR48217 at 6 mg/kg (once a week), whereas all control mice died. These findings point to the efficacy of FR48217 as an antiviral agent.

Acknowledgements

The authors are grateful to Dr. M. Okuhara, Fujisawa Pharmaceutical Co., for the kind gifts of FR41565 and FR48217, and to Dr. M. Ohashi, Department of Microbiology, Shiga University of Medical Science, for his helpful advice.

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