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Short Communication

Antiherpes activity of the immunomodulator OK-432, a streptococcal preparation, in immunosuppressed mice

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Summary

The antiviral activity of OK-432, an antitumor agent originating from Streptococcal preparations, against herpes simplex virus type 2 (HSV-2) was investigated in mice immunosuppressed by cyclophosphamide (CY). Intraperitoneal administration of OK-432 to mice 1 day after treatment with 200 mg CY/kg prevented death due to HSV-2 encephalitis in a dose-dependent manner. When the immunosuppressed mice were given OK-432 prior to HSV-2 infection, both by the intraperitoneal route, virus growth in the peritoneal cavity was significantly suppressed. Following with OK-432, the number of macrophages in immunosuppressed mice was increased to a significantly greater extent than the numbers of lymphocytes and polymorphonuclear leukocytes. The intrinsic antiviral activity of macrophages against HSV-2 as well as the natural killer (NK) activity against YAC-1 target cells was significantly enhanced by OK-432 in immunosuppressed mice.

OK-432; Immunomodulator; Herpes simplex virus; Immunosuppressed mice

During a primary HSV infection, the virus migrates from the infected area to a ganglion where it establishes a latent infection. The latent HSV has a tendency to

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be activated especially during immunosuppression, occasionally resulting in very severe disease. On rare occasions, HSV infections may be generalized in immunodeficient patients (Nahmias et al., 1976; Wheeler, 1975). HSV infections are also thought to be oncogenic, particularly in the development of cervical carcinoma (Rapp, 1973). Among a number of remedies tested for this recrudescent disease, acyclovir and foscarnet have been found to be therapeutically active (Alenius et al., 1982; Field et al., 1979). The present study was aimed at investigating the efficacy of an immunomodulator against HSV infection and establishing its mode of action.

OK-432 is a streptococcal preparation first developed in Japan and widely used as an antitumor agent (Hojo and Hashimoto, 1981; Kondo et al., 1975; Sakurai et al., 1972; Uchida and Hoshino, 1980). However, conclusive information as to how OK-432 enhances host-defense mechanisms against tumors is lacking. Possible mechanisms include the induction of interferon (IFN) (Saito et al., 1982) and tumor necrosis factor (TNF) (Yamamoto et al., 1985), and augmentation of NK activity (Oshimi et al., 1980; Uchida and Micksche, 1983). Since tumor patients are usually compromised in their immune system and vulnerable to virus infection, we investigated whether the immunomodulator OK-432 could restore the host-immune system and prevent HSV-2 spreading in immunosuppressed mice.

Female ddY mice bred for one week in a specific pathogen-free environment were used for experiments at 5 weeks of age. The UW strain of herpes simplex virus type 2 (HSV-2) grown in HEL (human embryonic lung) cells was stored at −80°C and used for the animal experiments. OK-432 (Chugai Pharmaceutical Co. Ltd., Tokyo, Japan) and CY (Shionogi Co. Ltd., Osaka, Japan) were dissolved in sterile physiological saline immediately before use. Groups of 10 mice were injected intraperitoneally (i.p.) with 200 mg CY/kg 4 days before virus infection. OK-432 diluted to various concentrations was then administered i.p. to CY-suppressed mice at various time intervals. After i.p. infection with 1×10^3 plaque-forming units (PFU) of HSV-2, the time of death was recorded daily for 14 days, and the percentages of surviving mice in experimental and control groups were compared statistically by the chi-square test. Peritoneal cells were obtained by lavage of the peritoneal cavity with Eagle's minimum essential medium (EMEM) according to the routine method described in a previous paper (Ikeda et al., 1985). The ratios of macrophages, polymorphonuclear leukocytes (PMN), and lymphocytes to the total peritoneal cell number were determined by microscopic observation after Giemsa staining. For assay of intrinsic antiviral activity of macrophages, peritoneal cells were harvested from normal or CY-treated immunosuppressed mice which had been given i.p. 100 μg OK-432 3 days previously. The activity against HSV-2 was assessed by determining virus growth in macrophages (Ikeda et al., 1985). Macrophages prepared at a cell concentration of 5×10^5/well were cultivated for 2 h at 37°C in a 24-well culture tray and infected with HSV-2 at a multiplicity of infection (MOI) of 0.1. After adsorption for 1 h, unadsorbed viruses were washed out twice with EMEM. The RPMI-1640 medium containing 10% fetal calf serum was added to the cells. After cultivation for 24 h at 37°C, the cells were harvested with a rubber policeman and disrupted by two freeze-thawing cycles. The virus ti-

ters in the cell homogenates were determined by a plaque assay on 3T3 monolayer cells. For assay of NK cell activity, the peritoneal cells were obtained from normal or CY-suppressed mice which were given i.p. 100 μg OK-432 3 days before the assay. NK activity was assessed by determining the radioactivity released from ^{51}Cr-labeled YAC-1 target cells (Ikeda et al., 1988). Briefly, the peritoneal cells were incubated for 4 h at 37°C with ^{51}Cr-labeled target cells in an effector:target ratio of 30:1. The cell mixtures were prepared in triplicate and the radioactivity released into the supernatant was counted in an autogamma scintillation spectrometer. The specific release of radioactivity was calculated according to the following formula: Specific lysis (%) = {(cpm of tested groups − cpm of the spontaneous release)/(cpm of the complete release − cpm of the spontaneous release)} × 100. The NK cell activity detected in the nonadherent fraction could be neutralized by treatment with anti-asialo GM_1 monoclonal antibody (Wako Chemicals, Co. Ltd., Tokyo).

When CY was administered i.p. to mice at a dose of 200 mg/kg 4 days before i.p. challenge with 10^3 PFU of HSV-2, mortality of CY-suppressed mice markedly increased in comparison with that of untreated mice. The mortality of immunosuppressed mice was reduced by preliminary administration of OK-432 between days 1 and 3 before HSV-2 challenge. As seen in Fig. 1, the protective activity of OK-432 was dose-dependent and most effective when 100 μg OK-432 was administered i.p. to CY-suppressed mice 3 days before HSV-2 infection. Growth of HSV-2 in the peritoneal cavity of CY-suppressed mice was enhanced 4-fold compared with that in untreated mice. Viral growth in the peritoneal cavity of CY-suppressed mice was markedly reduced following i.p. administration of 100 μg OK-432 3 days before HSV-2 infection (data not shown).

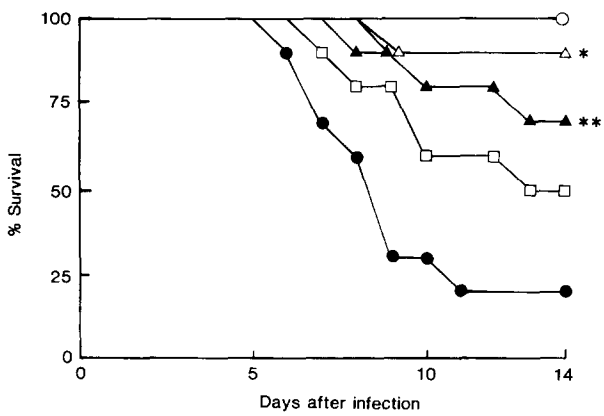

Fig. 1. Effect of OK-432 on the induction of resistance against HSV-2 in immunosuppressed mice. Groups of 10 mice were treated i.p. with 200 mg CY/kg 4 days previously and given i.p. different doses of OK-432 3 days before i.p. challenge with 1×10^3 PFU of HSV-2. ○, control; ●, CY-suppressed; △, CY-suppressed and treated with OK-432 (100 μg); ▲, CY-suppressed and treated with OK-432 (10 μg); □, CY-suppressed and treated with OK-432 (1 μg). $^*P < 0.005$, $^{**}P < 0.01$, as compared with CY-suppressed mice (χ^2 test).

TABLE 1

Effect of OK-432 on the recovery of peritoneal cells in immunosuppressed mice[a]

Treatment	Cell number ($\times 10^6$/mouse)			
	Total cells	Macrophages	PMNs	Lymphocytes
Non-treatment	5.62 ± 0.69	2.92 ± 0.36	0.06 ± 0.01	2.14 ± 0.25
CY(200 mg/kg, at −4d)	1.91 ± 0.23	0.82 ± 0.10	0.10 ± 0.01	0.96 ± 0.12
CY(200 mg/kg, at −4d)+ OK-432(100 μg, at −3d)	3.39 ± 0.16[b]	1.90 ± 0.09[b]	0.07 ± 0.01	1.36 ± 0.07[c]

[a]Peritoneal cells were harvested from mice treated i.p. with CY (200 mg/kg, at −4d) and OK-432 (100 μg, at −3d). The number of PC was counted using a hemocytometer, and cell population of the PC was determined microscopically by Giemsa staining.
[b]$P < 0.001$
[c]$P < 0.01$, as compared with CY-suppressed mice (Student t-test).

Total cell number and cell population in the peritoneal cavity of normal and immunosuppressed mice were examined as shown in Table 1. The total number of peritoneal cells (PC) in immunosuppressed mice was reduced 3-fold as compared to untreated mice. Upon administration of OK-432, the total PC number increased about 2-fold in immunosuppressed mice. The increase in macrophage number was greater than the increase in the number of lymphocytes and PMN.

Antiviral activity of peritoneal macrophages was assessed ex vivo by titration of viral growth in the macrophages of immunosuppressed mice. As shown in Table 2, HSV-2 replicated more readily in macrophages from CY-suppressed mice than in macrophages from untreated mice. However, when immunosuppressed mice had been treated with 100 μg OK-432 3 days before HSV-2 infection, viral replication was suppressed. Virus titers in macrophages from immunosuppressed mice treated with OK-432 were lower than virus titers in macrophages from immunosuppressed mice which had not been treated with OK-432, indicating that OK-432 restored antiviral activity of macrophages derived from CY-immunosuppressed mice.

The NK activities in peritoneal cells of three experimental groups (normal, CY-suppressed, and CY-suppressed OK-432-treated mice) were assessed by determining radioactivity released from ^{51}Cr-labeled YAC-1 target cells. The peritoneal cells were obtained from normal or CY-suppressed mice which had been given i.p. 100 μg OK-432 3 days previously. As shown in Table 3, NK activity was completely

TABLE 2

Effect of OK-432 on the intrinsic antiviral activity of macrophages of immunosuppressed mice[a]

Macrophage source	Virus titer (PFU/ml)	Ratio
Non-treatment	$(5.3 \pm 2.1) \times 10^2$	1.0
CY (200 mg/kg, at −4d)	$(1.3 \pm 0.6) \times 10^3$	2.5 (1.0)
CY (200 mg/kg, at −4d) + OK-432 (100 μg, at −3d)	$(1.6 \pm 0.6) \times 10^{2b}$	0.3 (0.1)

[a]The antiviral activity of peritoneal macrophages obtained from normal or CY-treated mice given i.p. 100 μg OK-432 was assessed by determining the virus growth in macrophages.
[b]$P < 0.001$, as compared with CY-suppressed mice.

TABLE 3

Effect of OK-432 on NK cell activity in immunosuppressed mice[a]

NK cell source	Specific lysis (%)
Non-treatment	2.0 ± 0.8
CY (200 mg/kg, at −4d)	− 0.9 ± 0.8
CY (200 mg/kg, at −4d) + OK-432 (100 μg, at −3d)	11.6 ± 0.9[b]

[a]The NK cell activity of peritoneal cells obtained from normal or CY-suppressed or CY-suppressed OK-432-treated mice was assessed by determining the radioactivity released from ^{51}Cr-labeled YAC-1 cells, as described in the text.

[b]$P < 0.001$, as compared with CY-suppressed mice.

abolished in peritoneal cells of CY-suppressed mice. However, the activity recovered if the CY-immunosuppressed mice had been treated with OK-432. The IFN titer obtained in the mouse peritoneal fluid 24 h after i.p. injection of 1×10^7 PFU of HSV-2 to normal and CY-suppressed mice was 190 and 135 U/0.1 ml, respectively. Administration of OK-432 by itself did not result in the production of peritoneal IFN (data not shown).

Buhles and Shifrine (1977) reported that administration of CY to mice resulted in marked reduction in numbers of various cell types including PMNs, blood monocytes and subpopulations of lymphocytes, and that injection of *Mycobacterium bovis* BCG or Freund complete adjuvant into CY-suppressed mice induced rapid restoration of cell number of peripheral blood granulocytes. The protective effect of OK-432 against HSV-2 infection in CY-suppressed mice is dependent on effective recruitment of blood monocytes into the peritoneal cavity and stimulation of their functional maturation in situ. Administration of OK-432 enhanced the intrinsic antiviral activity of macrophages and NK activity in immunosuppressed mice, suggesting that the activation of macrophages and NK cells plays an important role in the clearance of HSV-2 and HSV-2-infected cells in immunosuppressed mice.

In summary, our results indicate that OK-432 augments non-specific resistance against viral infection in immunocompromised mice. Since there was a reversed relationship between macrophage/NK cell functions and HSV-2 replication in mouse peritoneal cavity, OK-432 appears to be effective in suppressing HSV-2 infection in immunocompromised hosts.

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