EFFECT OF FK-506 ON REPLICATION OF HUMAN CYTOMEGALOVIRUS *IN VITRO*

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A new potent immunosuppressant, FK-506[1~3], has been found to have similar activities to cyclosporine[4~7]; suppression of mixed lymphocyte reactivity, interference with production of interleukin 2 and other lymphokines, *etc.* One major difference is that FK-506 is reported to have similar effects at approximately one tenth to one hundredth the concentration of cyclosporine. Human cytomegalovirus (HCMV) infection is a serious infection in immunocompromised patients including transplant recipients. This study was designed to examine the effect of FK-506 on HCMV *in vitro* and afford basic information for HCMV infection in the possible future use of FK-506, as a major immunosuppressant with/without cyclosporine.

Human embryonic lung (HEL) cells were grown and maintained in EAGLE's minimal essential medium supplemented with 10% and 3% fetal bovine serum, respectively. HCMV, herpes simplex virus (HSV) type I, and varicella-zoster virus (VZV) used were an AD169 strain, a Seibert strain, and a Kawaguchi strain.

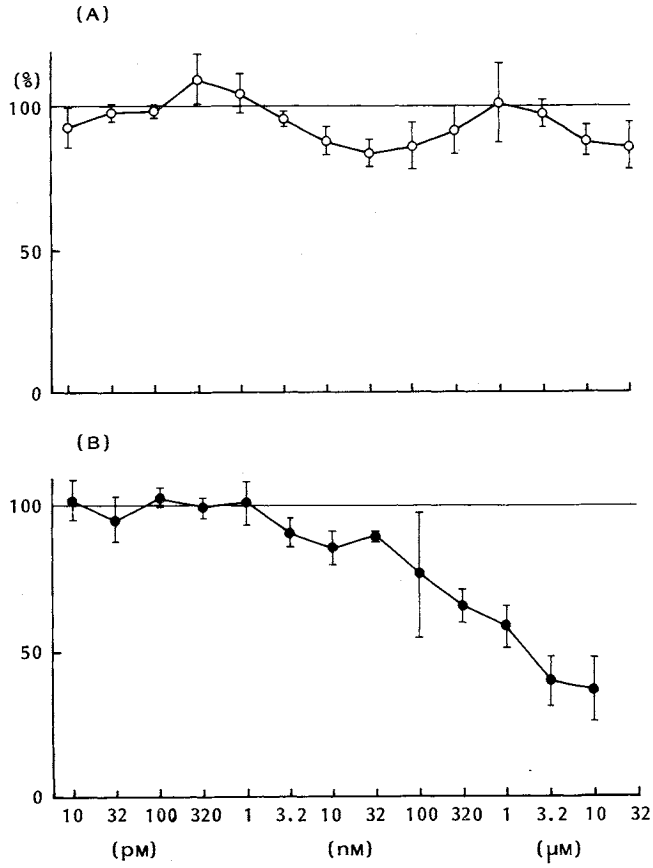
Monolayers of HEL cells in 60 mm plastic Petri dishes were inoculated with approximately 100 plaque forming units (PFU) of virus/0.2 ml. After incubation for 1 hour, 10 ml of nutrient methylcellulose medium (0.8%) and the required concentrations of FK-506 (kindly supplied by Fujisawa Pharmaceutical Co., Ltd., Japan) were added for HCMV and HSV. For VZV, 5 ml of maintenance medium was used instead of methylcellulose. After the appearance of cytopathic effects, the cells were fixed and stained, and the number of plaques was counted[8~10]. The number of plaques formed in the presence of FK-506 was compared with that formed in the absence of FK-506.

Intracellular virus growth (yield) was assessed by one step growth in cells treated with drugs[9~11]. HEL cells in 25 cm^2 plastic flasks were infected with 2 PFU of HCMV/cell for 1 hour. HCMV-infected cells were then washed three times with maintenance medium and incubated in this medium containing the indicated concentration of FK-506 for 3 days. Then the cells were washed three times, changed to 5 ml of fresh maintenance medium, frozen and thawed three times, and centrifuged at $1,500 \times g$ for 10 minutes. Serially diluted samples of the supernatants were inoculated onto HEL cells in 60 mm plastic dishes and the cells were overlaid with nutrient agarose or methylcellulose medium. After fixation and staining, the number of plaques was counted and intracellular virus yield in the presence of FK-506 was determined.

Fig. 1 shows the effect of FK-506 on the plaque formation of HCMV (Fig. 1A) and intracellular yield of HCMV (Fig. 1B). FK-506 had little effect on the plaque formation of HCMV at the concentrations examined but the sizes of plaques with high concentrations of FK-506 were smaller than those without FK-506. This suggested the possibility that FK-506 suppressed the intracellular growth of HCMV. As expected, FK-506 partially inhibited the yield of HCMV at concentrations more than 1 nM FK-506 as shown in Fig. 1(B). FK-506 had no effects on HSV or VZV (data not shown).

Although it is difficult to evaluate the effects on HCMV at the optimal concentration of FK-506 in transplantation because of limited studies of FK-506 on the immunosuppressive therapy in transplantation, plasma concentrations of FK-506 have been reported to range 0.5~12.4 ng/ml (0.6~15.1 nM)[12] and 0.2~23 ng/ml (0.24~28.0 nM)[13]. Those concentrations of FK-506 had no effect or slightly suppressive to HCMV replication *in vitro* as shown in Fig. 1. These results contrasted with the effects of cyclosporine on HCMV. Cyclosporine enhances replication of HCMV *in vitro* and the intracellular yield of HCMV in cells treated with 20~50 ng/ml (16.6~41.6 nM) of cyclosporine was enhanced 3.90 ($\pm$1.23: $n=5$) times of that in cells without cyclosporine[11]. Although FK-506 and cyclosporine exhibit similar effects on the immune system *in vitro* and *in vivo*, the effect on HCMV was dissociated, as observed in this study, *i.e.* enhancement of HCMV

Fig. 1. Effects of FK-506 on replication of HCMV.



(A) Effects of FK-506 on the plaque formation of HCMV. The number of plaques formed in the presence of FK-506 was expressed as the percentage of that in the absence of FK-506. Open circles and the ranges indicate the means and standard deviations of four experiments, respectively. (B) Effects of FK-506 on the intracellular growth (yield) of HCMV. Virus growth in the presence of FK-506 was expressed as the percentage of that in the absence of FK-506. Closed circles and ranges indicate the means and standard deviations of three experiments, respectively.

growth by cyclosporine and no effect or a little suppression by FK-506.

ALESSIANI *et al.*[14] reported that FK-506 treatment in liver transplant recipients showed a comparable incidence of bacterial and fungal infection but a significantly low incidence of symptomatic HCMV infection (0/20) compared with cyclosporine treatment (5/20). FK-506 accumulates in the lung of rats and its concentration reaches approximately 65 ng/ml (79.1 nM)[13], at which FK-506 suppressed to about 80% yield of HCMV in cells without FK- 506 treatment as shown in Fig. 1(B). This suggested that this concentration of FK-506 might reduce the total yield of HCMV to about 50% after three cycles of replication and lessen the pulmonary involvement by HCMV. Accumulation of FK-506 in the lung, which is a major target of HCMV infection, and suppressive effect of FK-506 on HCMV at higher concentrations might be beneficial to prevent HCMV disease in the lung. It is not clear whether effects of FK-506 and cyclosporine on HCMV replication *in vitro* directly reflect the incidence of severe HCMV infection in liver transplant recipients maintained on FK-506 and cyclosporine as observed, because the immunosupression maintained on FK-506 and cyclosporine may accompany different immune status in determining the susceptibility and severity of HCMV infection. Further clinical studies are required to evaluate the effect of FK-506 on the incidence and severity of HCMV infection in transplant recipients treated with FK-506 and/or cyclosporine.

In conclusion FK-506 had little or partial suppressive effect on replication of HCMV *in vitro* assessed by plaque formation and intracellular virus growth (yield) at the concentration used for immunosuppression *in vivo*. This made a good contrast to cyclosporine which enhanced replication of HCMV. Preliminary study[14] on HCMV infection in liver transplant recipients maintained on FK-506 and cyclosporine was discussed on the basis of the results of the effects of FK-506 and cyclosporine on HCMV replication *in vitro*.

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