Jpn. J. Vet. Res., **37**, 181–185 (1989)

CYTOTOXIC EFFECT OF ACYCLOVIR ON CULTURED MAMMALIAN CELLS TO WHICH HERPESVIRUS THYMIDINE KINASE GENE WAS INTRODUCED

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(Accepted for publication August 30, 1989)

The cytotoxic effects of acyclovir, which is a purine nucleoside analogue and is known as an antibiotic substance, were examined on three lines of rat skin fibroblast FR cells; normal FR cells, FRtk$^-$ cells which are deficient in the activity of thymidine kinase (tk) and FRtk$^-$HSVtk$^+$ cells which were prepared by introducing herpes simplex virus' tk gene to FRtk$^-$ cells. When FRtk$^-$ HSVtk$^+$ cells growing exponentially were incubated in the presence of acyclovir for 4 h, the surviving fractions of the cells decreased in a concertration-dependent manner. Whereas, decrease of the surviving fractions was almost indiscernible in both FR cells and FRtk$^-$ cells at the whole ranges of drug-concentrations tested. These results indicate that acyclovir is phosphorylated by the herpes simplex virus' tk and becomes toxic to FRtk$^-$HSVtk$^+$ cells. This also means that FRtk$^-$HSVtk$^+$ cells are useful for the investigation of the biological activity of nucleoside analogues.

Key words: acyclovir, herpesvirus thymidine kinase, cytotoxicity, phosphorylation, gene transfer.

INTRODUCTION

Ionizing radiation induces potentially lethal damage (PLD) into mammalian cells. The cells usually repair the PLD to survive. They die if the PLD is fixed. In some cases the cells mutate because of the misrepair of PLD. Therefore, the cellular PLD-repair system are thought to closely relate to mutation, carcinogenesis and aging.

If the drugs which prevent the PLD-repair are once found, several aspects of the mechanisms of PLD-repair will be elucidated by analyzing the action of drugs. Much effort has been made to search for such drugs. Especially, some nucleoside ana-

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logues which interfere with the metabolism of nucleic acids have been used as inhibitors of the PLD-repair (4, 7). Recently, it was reported that the cellular phosphorylation of nucleoside analogues was essential for their inhibitory action on the PLD-repair (3). However, many nucleoside analogues are usually hard to be phosphorylated by mammalian nucleside kinases, and do not serve as potent inhibitors of the PLD-repair.

Acyclovir among nucleoside analogues has the greatest inhibiting activity for herpes simplex virus type 1 (HSV-1) and an extremely low cytotoxicity to uninfected cells. This drug is found to be converted to the monophsphate form by the viral thymidine kinase (1, 2). The monophosphate form of acyclovir is subsequently converted to the diphosphate form and finally triphosphate derivatives which can serve as an anti-viral entity (1). Triphosphate derivative of acyclovir was also shown as an inhibitor of herpesvirus DNA polymerase (1).

In the present study, we introduced herpes simplex virus' tk gene in mammalian cells and examined the cytotoxic effects of acyclovir which is not usually phosphorylated by mammalian tk.

MATERIALS AND METHODS

Rat skin fibroblast FR cells and FRtk$^-$ cells, which are deficient in the activity of thymidine kinase (tk), were kindly supplied by Dr. A. Hakura and Dr. S. Kato (Research Institute for Microbial Diseases, Osaka University), respectively. The growth medium used for this experiment was Dulbecco's modified Eagle's medium (Nissui Co.) which was supplemented with 10% fetal bovine serum (Gibco). The HAT medium was prepared by adding 1×10^{-4}M hypoxantin, 4×10^{-5}M aminopterin and 1.6×10^{-5}M thymidine to the medium. The tk gene of herpes simplex virus (plasmid PHSV-106) for this experiment was purchased from Bethesda Research Laboratories (Gaithersburg, MD) and was identical to that of McKnight (6). Purified plasmid containing the tk gene was introduced into FRtk$^-$ cells by the calcium phosphate according to the method of Wigler *et al.* (8). The cells were fed by the growth medium for one day and then cultured for further 10 days using the HAT medium. After selection, the survived cells were cloned and designated as FRtk$^-$HSVtk$^+$. The activity of tk of FRtk$^-$HSVtk$^+$ cells or FRtk$^-$ cells was examined according to the method of Jamieson and Subak-Sharpe (5). The FR and FRtk$^-$ cells were subcultured in 60-mm plastic Petri dishes (Falcon) containing growth medium at 37°C in a humidified atmosphere of 95% air and 5% CO_2, whereas the FRtk$^-$HSVtk$^+$ cells were subcultured in the dishes containing HAT medium to suppress the appearance of revertant cells.

Acyclovir was purchased from Sumitomo Pharmaceuticals Co., Ltd. This drug was dissolved in Dulbecco's phosphate-buffered saline (PBS) and kept at −20°C until just before use. After the solution containing the drug was mixed with growth

medium, it was added to the cell cultures.

To obtain log-phase FR, FRtk$^-$ or FRtk$^-$HSVtk$^+$ cells, an appropriate number of cells were plated in the growth medium and incubated overnight. To test the cytotoxicity of acyclovir, the log-phase cells were incubated in the presence of this drug for 4 h at various concentrations. Following the treatment, the medium containing acyclovir was aspirated, and the cells were twice rinsed with PBS and incubated for 9 days in the growth medium for colony formation. The colonies were fixed with methanol and stained with 2% Giemsa solution. Only colonies composed of 50 or more cells were scored as having arisen from surviving cells. The surviving fractions were calculated by dividing the number of colonies of the acyclovir-treated cells by that of the colonies of untreated cells.

Results and discussion

Thymidine kinase activitiy of FRtk$^-$HSVtk$^+$ cells was 14.0 pmol dTMP formed / μg protein / 20 min, and was fairly higher than that of FRtk$^-$ cells (1.4 pmol dTMP formed / μg protein / 20 min). This result indicates that the FRtk$^-$HSVtk$^+$ cells, to which herpes simplex virus' tk gene was introduced, certainly expressed the tk activity.

Fig. 1 shows the cytotoxic effect of acyclovir to FR cells, FRtk$^-$ cells, and FRtk$^-$HSVtk$^+$ cells. When FRtk$^-$HSVtk$^+$ cells growing exponentially were incubated in the presence of various concentrations of acyclovir for 4 h, the cell-surviving fractions decreased 53% at 0.3 mM and 22% at 1.0 mM. However, the cell-surviving fraction was almost 100% when FR or FRtk$^-$ cells growing exponentially were incubated even in the presence of higher concentration of acyclovir (12 mM) for 4 h. These results indicate that acyclovir is phosphorylated by herpes simplex virus' tk and becomes toxic to FRtk$^-$HSVtk$^+$ cells, whereas this drug cannot be phosphorylated by tk of FR cell itself and therefore becomes no toxic to FR cells. Although it was reported that acyclovir was converted to the triphosphate derivative by herpes simplex virus' tk, and this derivative had ability to inhibit the herpesvirus DNA polymerase (1), the action of this drug to mammalian cells has not been fully elucidated because acyclovir is not phosphorylated by mammalian thymidine kinase. The present study suggests that acyclovir is phosphorylated in FRtk$^-$HSVtk$^+$ cells. This means that this cell line is useful in investigating the biological activities of nucleoside analogues in mammalian cells. On the other hand, because it was reported that the phosphorylation of the nucleoside analogues was one necessary condition for them to bring about the inhibiting activity of PLD-repair (3), the use of FRtk$^-$HSVtk$^+$ cells enables us to search for new types of nucleside analogues to inhibit PLD-repair. The finding of such nucleoside analogues would be useful for analyzing the mechanisms of PLD-repair.

ACKNOWLEDGMENT

The authors thank Miss Yoko Tamura for the technical assistance.

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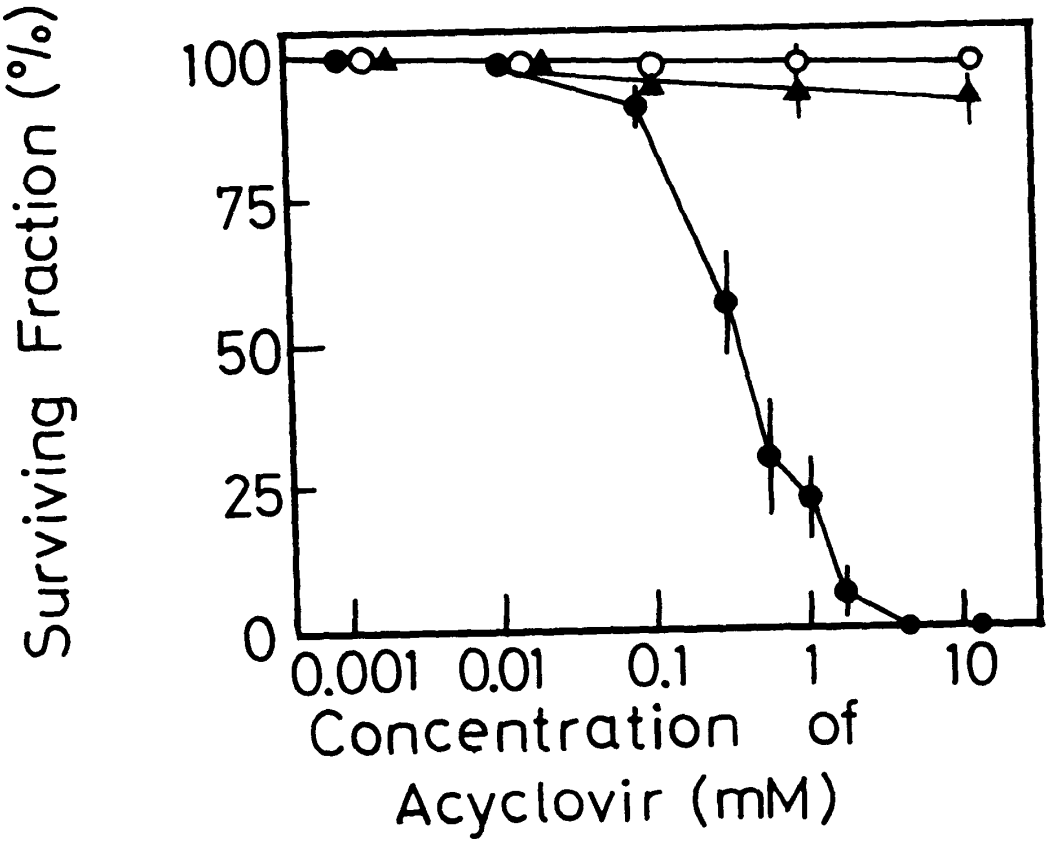

Fig. 1 The cytotoxic effects of acyclovir to FR cells (▲), FRtk$^-$ cells (○) and FRtk$^-$HSVtk$^+$ cells (●). Exponentially growing cells were incubated for 4 h in the presence of various concentrations of acyclovir. Each point is the mean from two repeated experiments. The error bars represent standard deviations. Multiplicity of the cells was 1.9.