Evaluation of cell cytotoxicity after ganciclovir treatment by radioiodinated IVDU

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1. Introduction

The herpes simplex virus type1 thymidine kinase(HSV1-tk) converts nontoxic nucleoside analogs such as ganciclovir into phosphorylated compounds that act as chain terminators and specially kill dividing cells (1). Unlike mammalian TK, HSV1-TK which is a non-specific nucleoside kinase, is encoded by a viral gene that is not present in normal mammalian cells (2).

Various radiolabelled nucleoside analogues are used as specific probes for HSV1-tk and can be freely transported across cell membranes. When phosphorylated by the tranduced HSV1-tk gene, the metabolites of probes subsequently accumulate within the transduced cells (3).

The aim of this study was to evaluate the inhibition of HSV1-tk transduced cells after Ganciclovir treatment on hepatoma cells (MCA-RH7777) infected a retroviral vector with the gene (HSV-tk) using pharmaceutical agent.

2. Methods and Results

2.1 synthesis and radioiodination

Radioiodinated IVDU was synthesized by the method of Morin et al. (1997), with minor modifications. The reaction was allowed to proceed for 15min at 25℃. After the reaction was over, the final product was purified by reverse phase HPLC on uBondapak C18 column (3.9 × 300mm, Waters, USA), using gradient elution with distilled water and acetonitrile. Retention time of radiolabeled compound was determined from UV and radioactivity detector (Raytest, Germany)data.

2.1. cell culture and MTT assay

MCA-RH7777(rat hepatoma cell) was obtained from the American Type Culture Collcetion(Rockville, MD, USA). MCA-tk was retrovirally transduced with the HSV1-tk gene. It was grown in the medium recommened by the supplier with 10% fetal bovine serum added.

MCA-tk cells were seeded at the number of 1.5×10^{4} Cells .well on 96well plates. On the following day, GCV was added to the final concentrations of 0- 75 ug/ml and the culture was incubated for 48hr.

Cell viability of MCA-tk cells had no effect for 24 hrs and 48hrs.

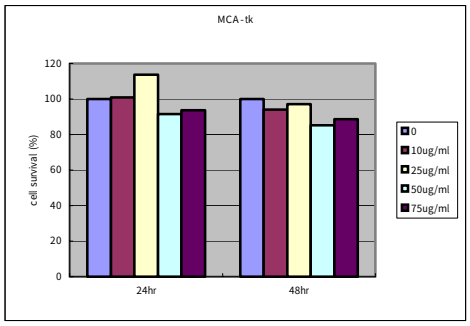

Figure 1. Cytotoxicity in MCA-tk cells with different concentrations (ug/ml) of GCV by MTT assay.

2.2. Flow cytometric analysis

The cells were treated with 0-75ug/ml GCV for 24hrs or 48hrs. Total cells were harvested and washed twice with PBS, and fixed in 70% ethanol overnight at 4 ℃. The cells were suspended in 1ml pf PBS containing 5ug/ml propidium iodide (PI) and 500ug/ml Rnase A at 37℃ for 30minutes and analyzed using cytometer.

Flow cytometry was employed for the analysis of cellular DNA contents and quantitation of cells undergoing apoptosis Analysis.

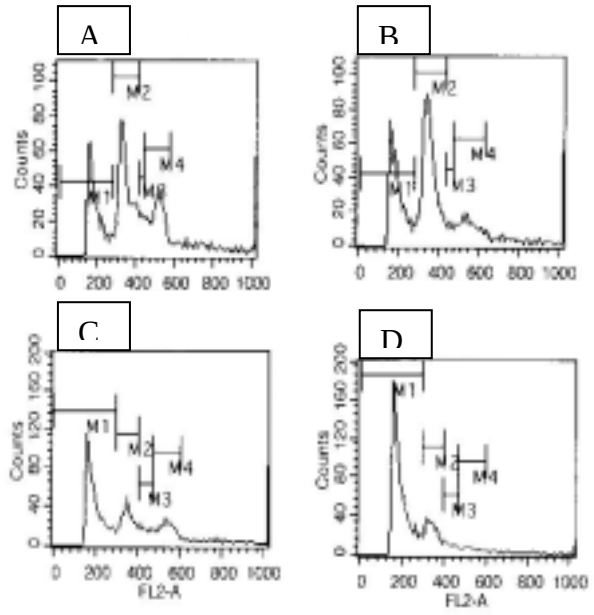

Figure 2. The images of flow cytometric analysis about MCA RH7777cells infected HSV1-tk gene.

A, B showed GCV treatment for 24hr and C,D for 48hr.

A,C : no GCV treatment .

B,D : 25ug/ml GCV treatment.

2.3. ^{125} *IVDU cellular uptake*

MCA-tk cells were assayed at fixed amount of radiopharmaceutical agent (^{125} IVDU) to moniter HSV1-tk gene expression for 24 and 48hr in the presence of different concentrations of GCV. the incubation time of the uptake was 4 hr in MCA-tk cells. IVDU uptake was decreased in a dose-dependent manner of GCV in MCA-tk cells.

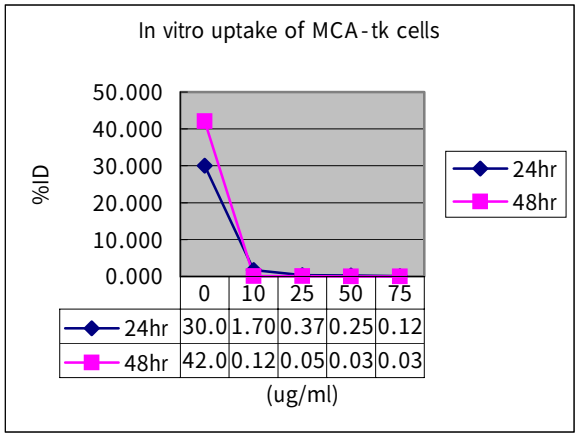

Figure 3. In vitro uptake of [^{125} I] IVDU

3. Conclusion

The radiolabed IVDU could be used as radiopharmaceutical agent to evaluate HSV1-tk gene expression and was more specific than MTT assay and FACS analysis .

REFERENCES

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