Biological Education of IVFRU and FIAU for HSV1-TK Reporter Gene Monitoring

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1. Introduction

The Herpes Simplex Virus Type1-thymidine kinase (HSV1-TK) system is a useful gene therapy monitoring method. HSV1-TK is one of the most widely used effector gene systems used for imaging gene expression, in association with its use as a reporter gene [1]. It has resulted the development of a number of radiolabled HSV1-TK substrates for the non-invasive detection of HSV1-TK expression [2]. In non-invasive imaging of the HSV1-TK system, many nucleoside derivatives have been developed as prodrugs for tumor proliferation imaging or as anti-viral drugs. Prodrug activation or sucide gene therapy has been shown to be successful in potentiating the therapeutic index by sensitizing genetically modified tumor cells to various prodrugs or enhancing the action of commonly used chemotherapeutic agents. The most studied prodrug activation approaches involve transfection of tumors with HSV1-TK gene [3]. (Z)-5-(2-iodovinyl)-2'-fluoro-2'-deoxyuridine (IVFRU) prossesses a 2'-fluoro substituent in the ribose configuration, is considered to protect IVFRU from enzyme mediated degradation in vivo. It is obviously potential substrates for HSV1-TK imaging [2]. 2'-Fiuoro-2'-deoxy-1-β-D-arabinofuranosyl-5-iodo-uridine (FIAU), an anticancer drug widely used in clinical practice, is an analogue of thymidine. In a series of studies using adenovirus vector for gene transfer described the appropriate combination of exogenously introduced HSV1-TK as a "marker/reporter gene" and radiolabelled FIAU as a "marker substrate/reporter probe" for monitoring gene therapy and gene expression [4].

2. Methods

2.1. Cell lines

The MCA cell line is a MCA RH7777 hepatoma cell line, and MCA-TK cells are a cell line derived from HSV1-TK expressind cells using aretroviral vector; both cell lines were gifts from Dr. Kwon of Molecular Oncology Laboratory [3].

2.2 Cellular uptake and nucleic incorporation

Cells were grown to 2×10^5 cells/well in 6-well culture plates and incubated 37℃ for 24 hr. [^{125}I]IVFRU and [^{125}I]FIAU were added to each well (1 μCi/2 ml). Thus the above mixture were incubated for 15, 30, 60, 120, 240, and 480 min at room temperature. The media were removed, the cells rinsed with PBS, and adherent cells were then lysed with 0.5 M perchloric acid (0.5 ml). The lysate was placed on ice for 30 min, vortex mixed and centifuged at 1500 rpm for 5 min in an Eppendorf microfuge. The supernatants was stored and the pellet washed with 0.5 M perchloric acid and again centrifuged 1500 rpm for 5 min. The radioactivity in the acid-insoluble fraction (nucleic acids and proteins) and acid-soluble fractions were determinded by gamma counting [2].

2.3 In vivo imaging of [^{131}I]FIAU in MCA and MCA-TK cells grafted mice

Planar imaging was performed. MCA and MCA-TK tumor cells (2×10^5 cells in 80 ul) were infected subcutanous into the right and left shoulders, respectively, of nude mice. After 7 weeks, the presence of the tumor mass was confirmed. One animals, carrier-added [^{131}I]FIAU (100 uCi/100 ul) were administered into the tail vein[1]. At 4 hr post-infection, radioactivity was measured with gamma camera.

3. Results

3.1 Cellular uptake and nucleic incorporation

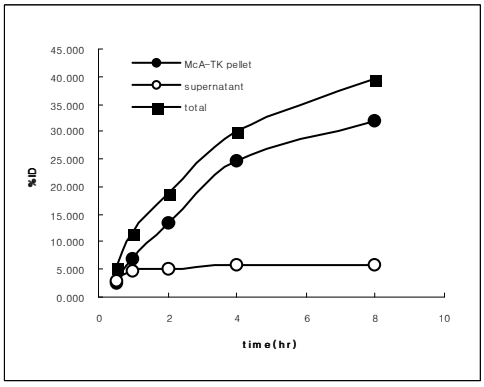

Figure-1. In vitro uptake of [^{125}I]FIAU in MCA-TK cell fractions.

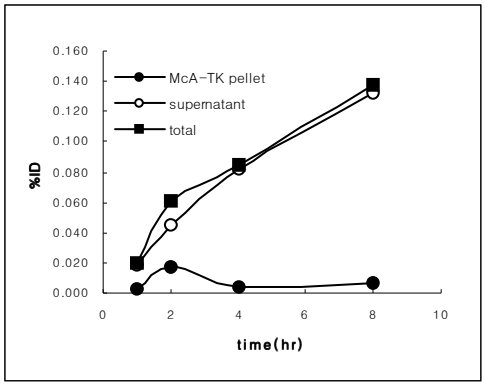

Figure-2. In vitro uptake of [^{125}I]IVFRU in MCA-TK cell fractions.

FIAU showed high uptake in the HSV1-TK cells. IVFRU also high uptake in the HSV1-TK cells than MCA cells.

FIAU has been shown to be a better substrates for HSV-1 TK. The current data provide evidence that FIAU are incorporated into the DNA of proliferating MCA-TK, with greater than 30% of the accumulated cellular radioactivity present in the acid-insoluble fraction (nucleic acid fraction) of MCA-TK cell lysate.

The relationship between uptake and activity concentration perhaps reflects some sort of saturable FIAU uptake mechanism (not shown).

IVFRU is also phosphorylated, but that is not metabolically elaborated into nucleic acids.

3.2 In vivo imaging of [^{131}I]FIAU in MCA and MCA-TK cells grafted mice

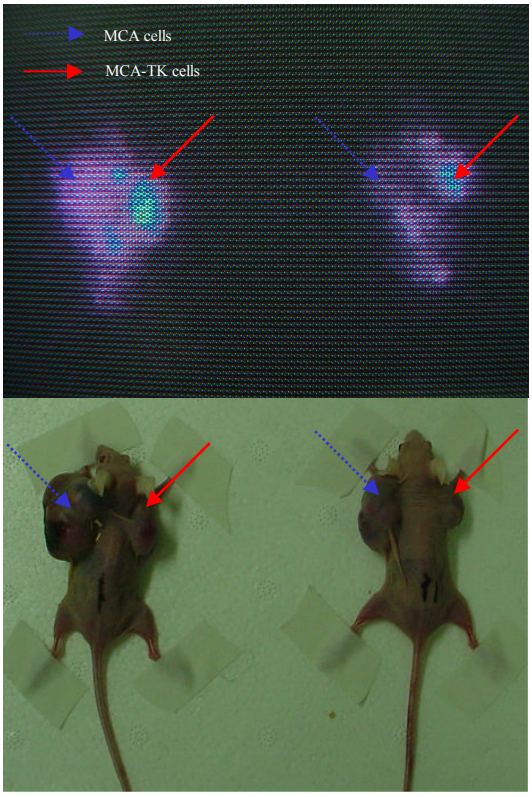

figure-3. Gamma camera images obtained 4h after I.V. administration of [^{131}I]FIAU to MCA and MCA-TK tumor-bearing mice.

The MCA-TK tumor was clearly visualised in mice that received administration [^{131}I]FIAU.

4. Conclusion

FIAU will be a better substrate than IVFRU for HSV1-TK gene expression monitoring. Specific imaging of HSV1-tk expression in animal tumor models were accomplished non-invasively with [^{131}I]FIAU and a clinical gamma camera system.

REFERENCES

[1] Tae Hyun Choi, Soon Hyuk Ahn, Hee-Chung Kwon, Chang Woon Choi, Ok Doo Awh, Sang Moo Lim. In vivo comparison of IVDU and IVFRU in HSV1-TK gene expressing tumor bearing rats. Appl Radiat Isot. 2004 Jan;60(1):15-21.

[2] Kevin W. Morin, Weili Duan, Lihua Xu, Aihua Zhou, Sameh Moharram, Edward E. Knaus, Alexander J.B. McEwan, Leonard I. Wiebe. Cytotoxicity and cellular uptake of pyrimidine nucleosides for imaging herpes simplex type-1 thymidine kinase (HSV-1 TK) expression in mammalian cells. Nucl Med Biol. 2004 Jul;31(5):623-30.

[3] Hackman T, Doubrovin M, Balatoni J, Beresten T, Ponomarev V, Beattie B, Finn R, Bornmann W, Blasberg R, Tjuvajev JG. Imaging expression of cytosine deaminase-herpes virus thymidine kinase fusion gene (CD/TK) expression with [^{124}I]FIAU and PET. Molecular Imaging. 2002 Jan;(1)-1: 36-42

[4] Win-Ping Deng1, Wen K. Yang, Wen-Fu Lai1, Ren-Shyan Liu, Jeng-Jong Hwang, Den-Mei Yang, Ying-Kai Fu, Hsin-Ell Wang. Non-invasive in vivo imaging with radiolabelled FIAU for monitoring cancer gene therapy using herpes simplex virus type 1 thymidine kinase and ganciclovir. European Journal of Nuclear Medicine and Molecular Imaging Vol. 31, No. 1, January 2004.

[5] Win-Ping Deng, Cheng-Chia Wu, Chien-Chih Lee, Wen K. Yang, Hsin-Ell Wang, Ren-Shyan Liu, Hon-Jian Wei, Juri G. Gelovani, Jeng-Jong Hwang, Den-Mei Yang, Ying-Kai Fu and Cheng-Wen Wu. Serial In Vivo Imaging of the Lung Metastases Model and Gene Therapy Using HSV1-tk and Ganciclovir. The Journal of Nuclear Medicine. 2006 May; 47(5):877-84.

[6] Hustinx R, Shiue CY, Alavi A, McDonald D, Shiue GG, Zhuang H, Lanuti M, Lambright E, Karp JS, Eck SL. Imaging in vivo herpes simplex virus thymidine kinase gene transfer to tumour-bearing rodents using positron emission tomography and [^{18}F]FHPG. Eur J Nucl Med. 2001 Jan;28(1):5-12.