Transactions of the Korean Nuclear Society Spring Meeting
Jeju, Korea, May 10-11, 2007

In vivo PET imaging with ^{18}F-FHBG of hepatoma cancer gene therapy using herpes simplex virus thymidine kinase and ganciclovir

TaeSup Lee a, JunYoup Kim, ByungSeok Moon, JooHyun Kang, Inho Song, HeeChung Kwon b, KyungMin Kim, GiJeong Cheon, ChangWoon Choi, SangMoo Lim
a Lab. of Nuclear Medicine, b Lab. of Molecular Oncology, Korea Institute of Radiological and Medical Sciences (KIRAMS), 215-4 Gongneung-Dong, Nowon-Gu, Seoul, 139-706, nobelcow@kcch.re.kr

1. Introduction

Monitoring gene expression *in vivo* to evaluate the gene therapy efficacy is a critical issue for scientists and physicians. Non-invasive nuclear imaging can offer information regarding the level of gene expression and its location when an appropriate reporter gene is constructed in the therapeutic gene therapy [1].

Herpes simplex virus type 1 thymidine kinase gene (HSV1-tk) is the most common reporter gene and is used in cancer gene therapy by activating relatively non-toxic compounds, such as acyclovir or ganciclovir (GCV), to induce cell death [2, 3].

In this study, we investigate the feasibility of monitoring cancer gene therapy using retroviral vector-transduced HSV1-tk and GCV, *in vitro* cellular uptake and *in vivo* animal studies, including biodistribution and small animal positron emission tomography (PET) imaging, were performed in HSV1-tk and luciferase (Luc)-transduced MCA-TK/Luc and enhanced green fluorescent protein (eGFP)-transduced MCA-eGFP hepatoma cell lines.

2. Methods and Results

2.1 Preparation of MCA-eGFP and MCA-TK/Luc cell

Before transfection of MFG-eGFP and MFG-TK-CMV-Luc, amphotropic retrovirus packaging cell line H29D (1x10^6 cells/dish) was seeded in 60 mm dish and cultivated for 24 h. After H29D cells were washed with PBS, eight ug of each plasmid transfected using WelFect-Q (WelGENE Inc.). After 72 h transfection, viral supernatant acquired and filtered with 0.45 um syringe filter.

Morris hepatoma cell line, MCA-Rh7777 (3x10^5 cells/well) was seeded in 6 well plate and cultivated for 24 h. Five hundreds ul of retroviral supernatant mixed with polybrene (8ug/ml) was added to MCA-Rh7777 washed with PBS and stirried 15 min interval for 2 h. After PBS washing in transfected cells, fresh 10% FBS DMEM was added. After 18 h transfection, MCA-Rh7777 was seeded with 100 cells per dish in 100mm dish, cultivated for 2 weeks and obtained clones. Established clone was trypsinized with Tripsin/EDTA and eGFP and luciferase expression were checked by fluorescence and luminescence activity using IVIS-200 (Xenogen, USA) We established MCA-eGFP and MCA-TK/Luc cell lines. MCA-eGFP cells had green fluorescence activity and MCA-TK/Luc cells had luminescence activity that determined by Bright-Glo kit (Promega, USA) (Fig. 1).

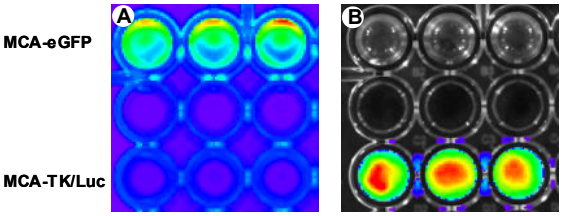

Figure 1. Fluorescence and bioluminscence of MCA-eGFP and MCA-TK/Luc cells. MCA-eGFP and MCA-TK/Luc cells (1X10^6 cell) were used in fluorescence and bioluminoscence assay. (A) Fluorescence activity (excitation and emission wavelength = GFP) and (B) bioluminescence activity were measured by IVIS-200.

2.2 In vitro uptake of ^{18}F-FHBG

For cellular uptake assay, cells of each cell line were trypsinised and grown overnight in 6-well culture plates (1×10^6 cells/2 ml/ well), and the medium was changed before experiment. ^{18}F-FHBG (2 μCi) was added to each well and incubated at 37°C for 30 min, 1 h and 2 h. Triplicates were performed at each time point. For ^{18}F-FHBG uptake assay, the supernatants were removed and the cells rinsed with 1 ml cold PBS. Then, cells in each well were harvested with 300 μl of trypsin-EDTA and washed twice with 2 ml PBS. Cellular uptake of ^{18}F-FHBG was determined by gamma counting in a Wallac 1470 Wizard gamma counter. Compared with the MCA-eGFP cells that contain no HSV1-tk gene, the HSV1-tk-transduced MCA-TK/Luc cells accumulated more radioactivity under all experimental conditions, and the ^{18}F-FHBG accumulation increased with time up to 2 h after exposure (Fig. 2).

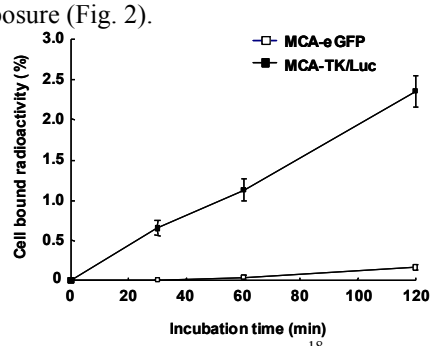

Figure 2. *In vitro* cellular uptake of ^{18}F-FHBG in MCA-eGFP and MCA-TK/Luc cells. Data represent Mean ± S.D.

After ganciclovir (GCV) treatment with 0, 0.1, 1, 10 ug/ml concentration, uptake of ^{18}F-FHBG (2 μCi) for 1 h was evaluated. Radioactivity in MCA-TK/Luc cell only was decreased as GCV dose dependent manners (Fig. 3).

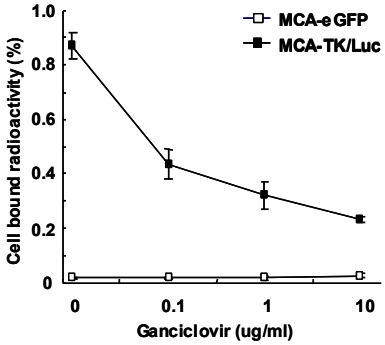

Figure 3. Change of ^{18}F-FHBG uptake in MCA-eGFP and MCA-TK/Luc cells treated with GCV. Data represent Mean ± S.D.

2.3 Biodistribution and In vivo PET imaging of ^{18}F-FHBG before and after ganciclovir treatment

MCA-eGFP and MCA-TK/Luc xenograft-bearing nude mouse was prepared by subcutaneouly injected tumor cells (2 X 10^6 cells/0.1ml) into left (MCA-eGFP) and right (MCA-TK/Luc) flank of nude mice. GCV treatment (50mg/Kg) started at 11 day post injection and GCV treated for 5 day(one per day, intraperitoneal; IP). For biodistribution experiments, animals were sacrificed 1 h after radiotracer administration. Organs were excised, weighed and assayed for ^{18}F radioactivity in a gamma counter to determine tissue uptake as a percentage of the injected radioactivity dose per gram of tissue (% ID/g). Three or four animals per group were used for biodistribution analysis.

PET Imaging was carried out on microPET-R4 (Concorde Microsystems Inc., Knoxville, TN) and acqusition time was 20 min. The microPET images were reconstructed with 2-dimensional ordered subset expectation maximization (OSEM) algorithm, after rebinning using Fourier rebining (FORE).

In biodistribution of ^{18}F-FHBG, radioactivity was selectively accumulated in MCA-TK/Luc tumor. The ratio of MCA-TK/Luc to MCA-eGFP was 5.69. After GCV treatment for 5 day, radioactivity was specifically reduced to 59.2% of pre-treatment in MCA-TK/Luc tumor and that ratio was decreased to 1.73 (Fig.4).

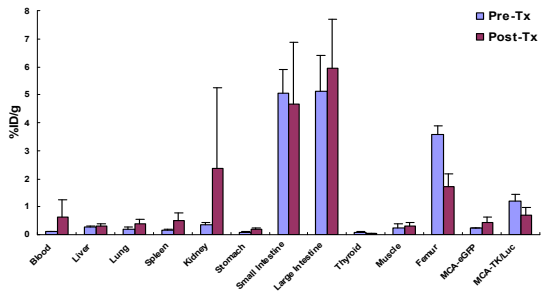

Figure 4. Change of ^{18}F-FHBG biodistribution in MCA-eGFP and MCA-TK/Luc tumor-bearing mice treated with GCV. Data represent Mean ± S.D.

In *in vivo* PET imaging, ^{18}F-FHBG was selectively localized in MCA-TK/Luc tumor and showed low uptake in MCA-eGFP tumor (Fig. 5).

After GCV treatment, Radioactivities accumulated in MCA-TK/Luc tumor was significantly decreased. This result closely correlated with biodistribution studies.

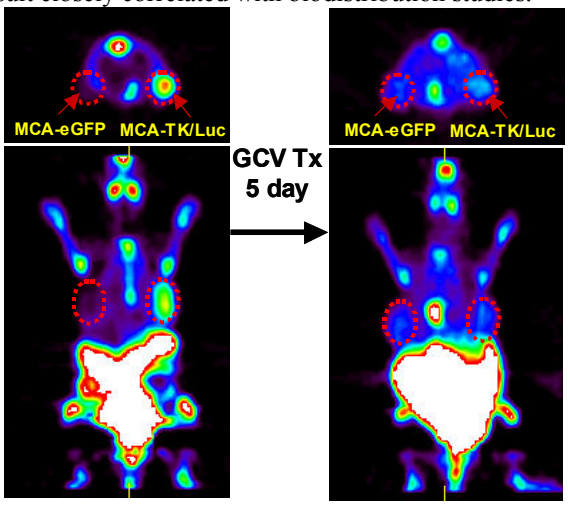

Figure 5. *In vivo* PET images of ^{18}F-FHBG in MCA-eGFP and MCA-TK/Luc tumor-bearing mice before (left panel) and after (right panel) GCV treatment.

3. Conclusion

The radiolabeled acycloguanosine derivative ^{18}F-FHBG exhibited as high specific accumulation in retroviral mediated HSV1-tk expressing cells both in cell culture and *in vivo*. For HSV1-tk stably transfected cells, ^{18}F-FHBG provided greater sensitivity over for hepatoma cell lines studied. The current study supports the view that ^{18}F-FHBG could be used as a reporter probe for HSV1-tk imaging when the gene is delivered as therapeutic approach.

^{18}F-FHBG is a promising marker substrate for *in vivo* monitoring of tumor expression of HSV1-tk and of therapeutic effects. The results support the view that FIAU may serve as an efficient and selective agent for monitoring of transduced HSV1-tk gene expression *in vivo* in clinical trials.

REFERENCES

[1] Tjuvajev JG, Stockhammer G, Desai R, Uehara H, Watanabe K, Gansbacher B, Blasberg RG. Imaging the expression of transfected genes in vivo. *Cancer Res* 1995; 55:6126-6132.

[2] Cheng YC, Grill SP, Dutschman GE, Nakayama K, Bastow KF. Metabolism of 9-(1,3-dihydroxy-2-propoxymethyl) guanine, a new anti-herpes virus compound, in herpes simplex virus-infected cells. *J Biol Chem* 1983; 258:12460-12464.

[3] Ido A, Nakata K, Kato Y, Nakao K, Murata K, Fujita M, Ishii N, Tamaoki T, Shiku H, Nagataki S. Gene therapy for hepatoma cells using a retrovirus vector carrying herpes simplex virus thymidine kinase gene under the control of human alphafetoprotein gene promoter. *Cancer Res* 1995; 55:3105-3109.