

## 專題研究計畫成果報告：

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計畫名稱：非侵入性分子核子醫學造影圖譜癌症基因治療之技術發展

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**Abstract.**

A new synthetic methodology was developed to synthesize and radiolabel FIAU (2'-fluoro-2'-deoxy-5-iodo-1- $\beta$ -D-arabinofuranosyluracil), the substrate for herpes simplex virus type 1 thymidine kinase gene (HSV1-tk). [ $^{131}$ I]-FIAU was performed by no-carrier-added synthesis process and lyophilized for "hot kit". The radio-labeling yield was over 95% and the radiochemical purity was more than 98%. The stability of [ $^{131}$ I]FIAU in lyophilized "hot kit" was better than in the normal saline solution. The shelf life of the final [ $^{131}$ I]FIAU "hot kit" product is as long as 3 weeks.

To examine the kinetics of  $^{131}$ I-FIAU and the possibility of utilizing for imaging of gene expression, cellular uptake of radiolabeled [ $^{131}$ I]-FIAU at different day's storage were performed in vitro in both murine sarcoma cell lines NG4TL4-STK and NG4TL4 over a period of 8 h. The NG4TL4-STK cells selectively accumulated the nucleotide analogue to the greatest extent for all different situations, with cellular radioactivity increasing up to 8-hour uptake. The kinetic profile of the uptake process in the lyophilized "hot kit" or the solution form is qualitatively similar.

In vivo experiment, FVB/N mice were inoculated subcutaneous with HSV1-tk(+) and tk(-) cells into the flank. Biodistribution studies were performed and the tumor/blood ratios were 2, 3.5, 8.2, and 386.8 at 1, 4, 8 and 24 h post-injection, respectively, for the HSV1-tk(+) tumors; and 0.5, 0.5, 0.7, and 5.4, respectively, for the HSV1-tk(-) tumors. Radiotracer clearance from blood was in 4-8hr and the difference between tumor(+) and other tissues, including tumor(-) was obvious. The highest activity accumulation ( $9.67\% \pm 3.89\%$  ID/g) in HSV1-tk(+) tumors was observed 24 h postinjection compared with HSV1-tk(-) tumors ( $0.48\% \pm 0.19\%$  ID/g). Planer gamma camera imaging showed HSV-tk(+) tumor regression after GCV treatment at day 4, and tumor nearly disappeared at day 7. Because of low non-target tissue uptake and image, new process of no-carrier-added synthesis [ $^{131}$ I]FIAU demonstrated as a perfect radiopharmaceutical for in vivo monitoring of gene transfer and expression.

**Key words:** [ $^{131}$ I]FIAU, non-invasive imaging, gene therapy, herpes simplex virus, thymidine kinase, and reporter gene

## Introduction

Monitoring gene expression in vivo to approach gene therapy is a critical issue for scientists and physicians. New 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 cassette[1]. Two approaches in noninvasive imaging development includes: (1) enzyme-mediated trapping by introducing reporter genes encoding enzymes that are able to catalyze radiolabelled substrates, subsequently trapped in transduced cells[2-5]; (2) receptor-ligand-mediated trapping on the cell surface[6, 7]. The expression of the reporter gene can be determined by using radiolabelled tracers imaged with a gamma camera, single photon emission computed tomography (SPECT) or positron emission tomography (PET) depending on the isotope used. If the reporter gene correlated with any therapeutic gene, the expression of the therapeutic gene could be monitored indirectly.

The ability to detect the localization, magnification and persistence of gene expression precisely and noninvasively would represent a big progress in the objective evaluation of gene therapy[8]. The expression of transfected gene could be demonstrated in vivo by using an appropriate combination of “reporter gene” and “reporter probe or marker substrate”[9, 10]. HSV-tk (herpes simplex viral thymidine kinase) is the most common reporter gene and its used in cancer gene therapy because of cell death by activation of relatively non-toxic prodrugs, such as ACV and GCV[11, 12]. In addition, lots of different radiolabelled nucleoside analogues are used as specific probes for HSV1-TK and can cross the cell membrane easily. When phosphorylated by the transduced HSV-tk gene, the metabolite of probes subsequently accumulated within the transduced cells. In contrast, mammalian thymidine kinase shows low affinity of phosphorylation for these probes, resulting in low levels of tracer accumulation in nontransduced cells. Therefore, specific accumulation of the radiolabelled nucleoside analogue in vivo reflects the expression of the transduced HSV1-tk gene.

FIAU, an anticancer drug widely used in clinic, is an analogue of thymidine [13, 14]. Saito et al. first demonstrated the selective uptake and autoradiographic imaging of herpes simplex virus infection in rat brain by using carbon-14 labelled FMAU (2'-fluoro-5-methyl-1- $\beta$ -D-arabinofuranosyl uracil)[15]. Tjuvajev et al. used HSV1-tk as a “marker gene” and radiolabelled [ $^{131}\text{I}$ ]FIAU and [ $^{124}\text{I}$ ]FIAU as a “marker substrate” for monitoring gene therapy, following assay by a clinical gamma camera system[16] and PET[17] respectively in an animal tumor model. Successful imaging showed specific accumulation of the tracer in the HSV1-TK-expressing tumor. In the literature, radiolabeled FIAU was prepared from (1) its unsubstituted precursor FAU (1-(2-deoxy-2-fluoro- $\beta$ -D-arabinofuranisyl)uracil) by direct iodination, and gave a 93% pure [ $^{131}\text{I}$ ]FIAU[16]. Non-carrier-added [ $^{124}\text{I}$ ]FIAU was prepared by reacting FAU with [ $^{124}\text{I}$ ]NaI and the synthesis yield was 95%[17]. (2) Using tributyltin as precursor, prepared FIAU from FTAU and got more than 90% of radioactivity in the radiochromatogram eluted as a single peak[18]. However, the simplicity and ready-to-use in the preparations of radiopharmaceuticals has also been considered to be important. Besides, detailed studies on the kinetics of [ $^{131}\text{I}$ ]FIAU have little to be performed[19].

In this study, we developed a new synthetic methodology using tributyltin as a precursor[20] to synthesize and radiolabel FIAU in lyophilized “hot kit”. The simplicity, high yield and high stability of no-carrier-added [ $^{131}\text{I}$ ]FIAU preparation has been implemented. The system is reliable, easy to set-up and operate. In addition, we examined the kinetics of [ $^{131}\text{I}$ ]FIAU and the possibility of utilizing for imaging of gene expression. In vitro cellular

uptake and in vivo animal model including biodistribution and planer gamma camera imaging studies were performed in both murine sarcoma cell lines NG4TL4-STK and NG4TL4. These results demonstrated [<sup>131</sup>I]FIAU can be used as a marker substrate for in vivo monitoring of gene transfer and expression.

*Note:* tk refers to the thymidine kinase gene and TK refers to the expressed enzyme

*The abbreviations used are:* HSV1-tk, herpes simplex virus type 1 thymidine kinase; FIAU, 2'-fluoro-2'-deoxy-1- $\beta$ -D-arabinofuranosyl-5-iodo-uridine; HPLC, high performance liquid chromatography; PET, positron emission tomography; ACV, acyclovir; GCV, ganciclovir

## Materials and methods

### *General*

2-Deoxy-2-fluoro-3,5-di-O-benzoyl-  $\alpha$  -D-arabinofuranose and uracil, along with the enzymes alcohol oxidase, catalase and alkaline phosphatase, were purchased from Sigma. Hexa-n-butylditin and bis(triphenylphosphine) were obtained from Strem. Hydrogen bromide (33% solution in acetic acid) and iodine monochloride and other chemicals were purchased from Merck.

The NMR spectra were recorded with a Bruker AC-300 Spectrometer at a proton frequency of 300 MHz and chemical shift were expressed in ppm. Thin layer chromatography was conducted using an imaging scanner (System 200, Bioscan). High-performance liquid chromatography was conducted using Waters Model 600, Waters Model 600E pumps, a Waters Model 486 tunable UV detector along with a radioisotope detector (Flow Count Detector FC-003, Capintec, Bioscan). Data were collected and analyzed using a computer program (CSW, version 1.7, DataApex Ltd). The radiochemical yields reported are those obtained at the end of synthesis.

### *Preparation of 2-Deoxy-2-fluoro-3,5-di-O-benzoyl- $\alpha$ -D-arabinofuranosyl Bromide (2a)*

Using a modification of a reported procedure (Huges, 1995)[21], we dissolved two grams (4.3 mmol) of 2-deoxy-2-fluoro-1,3,5-tri-O-benzoyl-  $\alpha$  -D-arabinofuranose (**1a**)(Fig.1) in 50 mL dry dichloromethane. Under a nitrogen atmosphere, hydrogen bromide (33%) in acetic acid (1.25mL) was slowly added to **1a**. The mixture was stirred at room temperature overnight. After cooling to ambient temperature, the solvent was removed in vacuo. The crude product was purified by silica gel chromatography using 1:14 ethyl acetate: hexane to yield 1.65 g of the bromo derivative **2a** (91%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  8.2 ~ 7.4 (m, 10H, ArH), 6.6 (d, 1H, C'<sub>1</sub>-H; J<sub>F-H</sub> =12 Hz), 5.6 (d, 1H, C<sub>2</sub>-H; J<sub>F-H</sub> =50 Hz), 5.5 (dd, 1H, C<sub>3</sub>-H; J<sub>F-H</sub> =22 Hz , J =3 Hz), 4.75 (m, 3H, C<sub>4</sub>-H, C<sub>5</sub>-H<sub>2</sub>).

### *Preparation of 2,4-Bis-O-(trimethylsilyl)uracil (2b)[22]*

A mixture of uracil (**1b**) (500 mg, 4.46 mmol ), ammonium sulfate (0.59g, 4.46 mmol ) and hexamethyldisilazane (10mL) was refluxed for 4 hr. After cooling to room temperature, the clear solution was evaporated under reduced pressure to give a thick oil **2b**, which was used without any further purification.

### *Preparation of 1-(3,5-Di-O-benzoyl-2-deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl uracil (3)*

Compound **2a** (1.7 g, 4.3 mmol ), dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15mL), was tubing transferred into a flask containing compound **2b** in CH<sub>2</sub>Cl<sub>2</sub> (15mL). The mixture was heated to reflux overnight. The solvent was removed in vacuo to give a white solid residue. The crude product was purified by silica gel chromatography using 1:1 ethyl acetate: hexane to give 1.36 g of the

white uracil derivative **3** (75%).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  8.41 (br s, 1H, NH), 8.04 (m, 4H, ArH), 7.66 ~ 7.41 (m, 7H, ArH;  $\text{C}_6\text{-H}$ ), 6.31 (dd, 1H,  $\text{C}_1\text{'-H}$ ;  $J_{\text{F-H}} = 21.7\text{Hz}$ ,  $J = 2.7\text{Hz}$ ), 5.67 (dd, 1H,  $\text{C}_5\text{'-H}$ ;  $J = 17.2\text{Hz}$ ,  $J = 2.2\text{Hz}$ ), 5.62 (dd, 1H,  $\text{C}_3\text{'-H}$ ;  $J_{\text{F-H}} = 18.5\text{Hz}$ ,  $J = 2.7\text{Hz}$ ), 5.31 (dd, 1H,  $\text{C}_2\text{'-H}$ ;  $J_{\text{F-H}} = 50.4\text{Hz}$ ,  $J = 2.0\text{Hz}$ ), 4.75 (m, 2H,  $\text{C}_5\text{'-H}_2$ ), 4.50 (m, 1H,  $\text{C}_4\text{'-H}$ ).

*Preparation of 1-(3,5-Di-O-benzoyl-2-deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl)-5-iodouracil (4)*

A sample of **3** (260mg, 0.58mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (50mL), and ICI (200mg) was added. The solution was heated to reflux for 6 h and the solvent was removed under reduced pressure. The crude product was washed with  $\text{H}_2\text{O}$  (50mL x 2), dried, and evaporated. A 265.6 mg (78%) white solid was obtained by silica gel chromatography using 1:1 ethyl acetate: hexane.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  8.43 (br s, 1H, NH), 8.04 (m, 5H, ArH), 7.63 ~ 7.43 (m, 6H, ArH;  $\text{C}_6\text{-H}$ ), 6.29 (dd, 1H,  $\text{C}_1\text{'-H}$ ;  $J_{\text{F-H}} = 21.7\text{Hz}$ ,  $J = 2.8\text{Hz}$ ), 5.60 (dd, 1H,  $\text{C}_3\text{'-H}$ ;  $J_{\text{F-H}} = 18.2\text{Hz}$ ,  $J = 2.6\text{Hz}$ ), 5.35 (dd, 1H,  $\text{C}_2\text{'-H}$ ;  $J_{\text{F-H}} = 52.8\text{Hz}$ ,  $J = 2.0\text{Hz}$ ), 4.80(m, 2H,  $\text{C}_5\text{'-H}_2$ ), 4.50 (m, 1H,  $\text{C}_4\text{'-H}$ ).

*Preparation of 1-(3,5-Di-O-benzoyl-2-deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl)-5-tributylstannyluracil (5)*

A mixture of 146.7mg (0.25 mmol) of **4**, and 261 mg (0.45 mmol) hexabutylditin, and 10mg of bis(triphenylphosphine)palladium dichloride was dissolved in 7.5 mL of dry dioxane. After the mixture was refluxed for 6 h under a nitrogen atmosphere, the dioxane was removed by rotary evaporation. The light green precipitate was filtered through celite. The filtrate was adsorbed onto silica gel and purified by silica gel chromatography (1:3 ethyl acetate : hexane to 100 % ethyl acetate) to a dry solid to yield 98.4 mg of **5** (53% yield).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  8.89 (s, 1H, NH), 8.04 ~ 7.34 (m, 11H, ArH;  $\text{C}_6\text{-H}$ ), 6.33 (dd, 1H,  $\text{C}_1\text{'-H}$ ;  $J_{\text{F-H}} = 21.5\text{Hz}$ ,  $J = 2.6\text{Hz}$ ), 5.65 (dd, 1H,  $\text{C}_3\text{'-H}$ ;  $J_{\text{F-H}} = 18.8\text{Hz}$ ,  $J = 2.8\text{Hz}$ ), 5.33 (dd, 1H,  $\text{C}_2\text{'-H}$ ;  $J_{\text{F-H}} = 52.8\text{Hz}$ ,  $J = 2.0\text{Hz}$ ), 4.70(m, 2H,  $\text{C}_5\text{'-H}_2$ ), 4.48 (m, 1H,  $\text{C}_4\text{'-H}$ ), 1.37 ~ 0.78 (m, 27H,  $\text{SnBu}_3$ ).

*Preparation of 5-Tributylstannyl -1-(2-deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl)uracil (FTAU) (6)*

The above product **5** (52.7mg) was added to 10 mL of conc.  $\text{NH}_4\text{OH}$  :  $\text{MeOH} = 80:20$ . The mixture was stirred for 24 h and the solvent was removed under reduced pressure. The crude oil product was purified by silica gel chromatography using 11:1 chloroform: hexane giving 33 mg of **6** (87%).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  7.48 (d, 1H,  $\text{C}_6\text{-H}$ ;  $J = 2.0\text{Hz}$ ), 6.24 (dd, 1H,  $\text{C}_1\text{'-H}$ ;  $J_{\text{F-H}} = 18\text{Hz}$ ,  $J = 3.9\text{Hz}$ ), 5.04 (dm, 1H,  $\text{C}_2\text{'-H}$ ;  $J_{\text{F-H}} = 50\text{Hz}$ ), 4.29 (dm, 1H,  $\text{C}_3\text{'-H}$ ;  $J_{\text{F-H}} = 16\text{Hz}$ ), 3.92 ~ 3.70(m, 3H,  $\text{C}_4\text{'-H}$ ;  $\text{C}_5\text{'-H}_2$ ), 1.54 ~ 0.85 (m, 27H,  $\text{SnBu}_3$ ).

*No-Carrier-added Synthesis [ $^{131}\text{I}$ ]-FIAU from Precursor FTAU*

No-carrier-added 5- $^{131}\text{I}$ -Iodo-1-(2'-deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl) uracil ( $^{131}\text{I}$ -FIAU) was synthesized from its organotin precursor (Fig. 2). 100  $\mu\text{L}$  of oxidizing agent ( $\text{H}_2\text{O}_2:1\text{N HCl}:\text{H}_2\text{O} = 4:1:95$ ) was added to a 300  $\mu\text{L}$  V-vial coated with 15  $\mu\text{g}$  of 5-tributylstannyl-(2'-deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl)uracil (FTAU) and containing 20  $\mu\text{L}$  ethanol and 0.1~1 mCi sodium [ $^{131}\text{I}$ ]iodide. The reaction mixture was vortexed intermittently. After 8 min, the mixture was frozen in liquid nitrogen, and then lyophilized with a vacuum system (equipped with a charcoal absorber) for about 1 hr to give the final product as a "hot kit". The lyophilized [ $^{131}\text{I}$ ]FIAU "hot kit" was redissolved in ethanol and the radiochemical purity was determined using TLC and HPLC. Thin layer chromatography was performed on TLC aluminium sheet (Silica gel 60F<sub>254</sub>, MERCK), using ethyl acetate/ethanol (90/10, v/v) as the mobile phase. Chromatograms were recorded using an imaging scanner (system 200, BIOSCAN). HPLC analysis was performed on a reversed-phase column (RPR-1,

Hamilton), using methanol/water (50/50, v/v) as the eluant at a flow rate of 1 mL / min. A UV detector (tunable absorbance detector 486, Waters) and a radiodetector (CAPINTEC, BIOSCAN) were used to analyze the eluate. Data were collected and analyzed using computer software (CSW, version 1.7, DataApex Ltd). The lyophilized [ $^{131}\text{I}$ ]FIAU product, dissolved in physiological saline and eluted through a 0.22  $\mu\text{m}$  apyrogenic disk, was ready for biological or clinical application. In this modified radio-labeling method, unreacted [ $^{131}\text{I}$ ]iodide (in form of  $^*\text{I}_2$  in the presence of oxidizing agent), HCl, solvents (ethanol and  $\text{H}_2\text{O}$ ) and oxidizing agent ( $\text{H}_2\text{O}_2$ ) were all removed, only [ $^{131}\text{I}$ ]FIAU and a tiny amount of FAU (less than 10  $\mu\text{g}$ ) were left in the final “hot kit” product after lyophilization, hence no further purification was needed.

The theoretical specific activity of [ $^{131}\text{I}$ ]-FIAU prepared from a no-carrier-added synthesis can be determined from the equation:

$$\text{Specific activity} = Av / (R \times t_{1/2}) \quad (\text{A})$$

Where  $Av$  is Avogadro's number  $6.02 \times 10^{23}$ ,  $R$  is the conversion factor  $3.7 \times 10^{10}$  Bq/Ci,  $t_{1/2}$  is the physical half-life of the radionuclide in sec. The theoretical specific activity for [ $^{131}\text{I}$ ]-FIAU is  $\sim 2 \times 10^7$  Ci/mol.

#### *Stability of No-Carrier-added Synthesis [ $^{131}\text{I}$ ] FIAU*

In order to evaluate the stability of no-carrier-added synthesis [ $^{131}\text{I}$ ] FIAU, the radiochemical purity of [ $^{131}\text{I}$ ]FIAU in the lyophilized “hot kit” product and in the normal saline solution were determined at 1, 2, 3, 5, 7, 10, 14, 21, 28 days after preparation.

#### *Cellular Uptake of No-Carrier-added [ $^{131}\text{I}$ ]-FIAU*

Two murine cell lines (NG4TL4 and NG4TL4-STK) were used to evaluate no-carrier-added synthesis [ $^{131}\text{I}$ ]-FIAU from precursor FTAU. The NG4TL4-STK cell line, derived from parental NG4TL4 sarcoma cell, has been described previously[23]. The NG4TL4 sarcoma cells were infected with packaged virions of a recombinant retroviral vector constructed to contain *HSV-1-tk* gene[24-28] that carries its own promoter and *neo*<sup>R</sup> gene that carries simian virus 40 early promoter. NG4TL4 and NG4TL4-stk cells were cultured in MEM supplemented with 10% FBS, 1% penicillin-streptomycin-1% L-glutamine in a humidified atmosphere with 5%  $\text{CO}_2$  at 37°C.

For cellular uptake assay, cells of each cell lines were trypsinized and grown over night in 24-well culture plate ( $3 \times 10^5$  cells/ 0.5 ml/ well) and medium was changed before experiment. No-carrier-added [ $^{131}\text{I}$ ]-FIAU ( 0.08 $\mu\text{Ci}$ / 0.5 ml/ well) were added to each well and incubated at 37°C for 6 varying times (15 min, 30 min, 1 h, 2 h, 4 h, 8 h) incubation. Triplicates were performed at each time point for uptake assay. After exposure to [ $^{131}\text{I}$ ]-FIAU, the supernatants were removed and the cells rinsed with 200  $\mu\text{l}$  HBSS. Then the cells were trypsinized with 100  $\mu\text{l}$ /well Trypsin-EDTA, and washed twice with 150  $\mu\text{l}$  HBSS. Cellular uptake was determined by gamma counting in a Wallac 1470 Wizard gamma counter.

#### *Biodistribution of FIAU*

The syngeneic FVB/N inbred strain mice were used to perform biodistribution studies. No-carrier-added [ $^{131}\text{I}$ ]-FIAU (0.01 mCi/animal) was injected i.v. 10 days after NG4TL4 or NG4TL4-STK cells ( $1 \times 10^5$  cells) were inoculated subcutaneously into the flank of female FVB/N inbred strain mice. Groups of three animals were killed at 1, 4, 8, and 24 h postinjection. Dissected tissue of interest was harvested, washed, weighted and quantified along with injection standards using a Wallac 1470 Wizard gamma counter.

#### *Planar Imaging*

Planar imaging was performed also in the syngeneic FVB/N inbred strain mice bearing NG4TL4-STK tumor. NG4TL4-STK cells ( $1 \times 10^5$  cells) were inoculated subcutaneously into

the flank of female FVB/N inbred strain mice, which all developed around 10 mm in diameter and palpable tumors. After the injection of [ $^{131}\text{I}$ ]-FIAU (0.01 mCi/animal) via the tail vein, static planar images were obtained from anaesthetized animals under a pinhole collimator on a \*\*\* gamma camera (\*\*\*) with .

## Results

### *The Preparation and Stability of no-carrier-added Synthesis [ $^{131}\text{I}$ ]-FIAU*

The final product, lyophilized [ $^{131}\text{I}$ ]FIAU “hot kit”, was redissolved in ethanol and the radiochemical purity was determined using TLC and HPLC. Thin layer chromatography showed the  $R_f$  value of [ $^{131}\text{I}$ ]FIAU was 0.82~0.83 (Fig.3a). The retention time of [ $^{131}\text{I}$ ]FIAU was 12.91 min by HPLC analysis (Fig.3b), the same as that obtained from the cold FIAU standard. The labeling yield was more than 95% and the radiochemical purity was more than 98% (in average from more than 10 runs). The stability of no-carrier-added synthesis of [ $^{131}\text{I}$ ]FIAU in the lyophilized “hot kit” product showed significantly more stable than in the normal saline solution by TLC assay. The shelf life of the final [ $^{131}\text{I}$ ]FIAU “hot kit” product is as long as 3 weeks (table 1).

### *Cellular Uptake of No-Carrier-added [ $^{131}\text{I}$ ]-FIAU*

Cellular uptake for evaluating the stability of no-carrier-added synthesis of [ $^{131}\text{I}$ ]-FIAU was performed in two cell lines of murine. Figure 4 showed *In vitro* cellular uptake of [ $^{131}\text{I}$ ]-FIAU in the lyophilized “hot kit” at 1 day (a), 7 day (b), 28 day (c), and in the solution form at 28 day (d) in both murine sarcoma cell lines NG4TL4-STK and NG4TL4 over a period of 8 h.

In contrast to the NG4TL4 cells that lack HSV1-TK expression, the NG4TL4-STK cells selectively accumulated the nucleotide analogue to the greatest extent for all different situations, with cellular radioactivity increasing up to 8 hour after exposure. The kinetic profile of the uptake process in the lyophilized “hot kit” or between the lyophilized and the solution form is qualitatively similar.

### *The kinetic of Biodistribution and tumour/blood ratio*

The biodistribution of  $^{131}\text{I}$ -FIAU content in several tissues was determined by *ex vivo* counting. FVB/N mice were inoculated subcutaneous with *HSV1-tk*(+) and *tk*(-) cells into the flank. Biodistribution studies were performed at 1, 4, 8 and 24 h post-injection (Fig.5). As shown in figure 5b, the NG4TL4-STK tumor tissues retained the highest level than all other organs at any time course after i.v. administration. The difference reached the maximum at 24 hr. post-injection [ $^{131}\text{I}$ ]FIAU. At that time, NG4TL4-STK tumor retained  $^{131}\text{I}$ - radioactivity (9.67 %ID/g) than non-transduced NG4TL4 tumor (0.48 %ID/g). Tracer clearance from blood was in 24hr and the highest activity accumulation in *HSV1-tk*(+) tumors was observed 24 h p.i.

The kinetic of tumour/blood ratios at 1, 4, 8, and 24 h showed the kinetic accumulation level in 2.0, 3.5, 8.2, and 386, respectively, for the *HSV1-tk*(+) tumors, and 0.6, 0.5, 0.7, and 5.4, respectively, for negative control tumors (Table 2). The kinetics of tissue clearance was also shown in figure 6. Compared with blood, liver and tumor(*tk*-) tissue, NG4TL4-STK tumor(*tk*+) showed high and lasting accumulation during the period (Fig. 6A). In blood, liver and NG4TL4 tumor(*tk*-) only showed biphasic elimination characteristics (Fig. 6B).

### *Tumor Regression and PET Studies*

Low non-target tissue uptake of radioactivity and obvious tumor:blood ratios suggest that the NG4TL4-STK tumor could be imaged scintigraphically with [ $^{131}\text{I}$ ]FIAU. Planer gamma camera imaging after intravenous injection of [ $^{131}\text{I}$ ]FIAU clearly reflected the transduced tumor (Fig. 7). Besides, mice imaging showed non-specific uptake in the stomach and thyroid.

The image of the same animal, after GCV treatment at day 4, showed HSV-tk(+) tumor regression and tumor nearly disappeared at day 7. These results demonstrated [131I]FIAU can be used as a reporter gene for in vivo monitoring of gene transfer and expression

## Discussion

More and more gene therapy protocols and clinical trials are in progress, thus, developing non-invasive methods to determine the success of gene transfer and gene expression in vivo is more important. Using the HSV-tk gene as a reporter gene and [131I]FIAU as a marker substrate is well investigated. The aim of this study was to improve the suitable radiolabeled probe to be used as a marker substrate for cells transduced with HSV-tk gene during in vivo gene therapy.

To reach the simplicity, faster, and easier in the preparations of radiopharmaceuticals, we referred to Dougan et. al. 1994 and developed a new synthetic methodology. We selected the tributyltin as a precursor to synthesize and radiolabel FIAU as lyophilized “hot kit” because of less toxicity of the tributylstannyl group compared with the trimethyl group[29, 30]. The convenience and high yield of [131I]FIAU preparation and the stability of [131I]FIAU are better than before.

The new I-131 labeling method using tributyltin as precursor and H<sub>2</sub>O<sub>2</sub> as oxidant has lots of advantages: (1) Acid and oxidant can use to remove extra I-131 without any other purification steps. The lyophilized product only have [I-131]FIAU and little FAU. (2) This method enhanced the stability of the final [<sup>131</sup>I]FIAU “hot kit” product and the shelter life of the product as long as 3 weeks. (3) The new method is clearance, rapid, high yield and efficiency due to only 8 minutes preparation of [I-131]FIAU. The radio-labeling yield was over 95% and the radiochemical purity was more than 98%, which were higher than Vaidyanathan et. al. (1998, 90%)[18], and Tjuvajev et al. (1996, 93%)[16].

Biodistribution studies were performed at different time postinjection to examine the kinetic of <sup>131</sup>I-FIAU accumulation in vivo. As shown in figure 5b, the NG4TL4-STK tumor tissues retained the increased accumulation follow by the time course and reach the maximal at 24 h p.i. In contrast, all other organs remained at low level up to 24 h p.i. High radioactivity accumulation was observed in the kidneys and the blood in the early phase (1, 4 hr) cause to renal excretion. Tracer clearance from blood was in 24hr, and extremely low radioactivity accumulation in brain was observed during the period due to the blood-brain barrier.

The kinetic of tissue/blood ratios at 1, 4, 8, and 24 h showed the kinetic accumulation level in *HSV1-tk*(+) tumours. Compared with the HSV1-TK-expressing tumours, there was low tracer accumulation in all the other organs, including the *HSV1-tk*-negative tumours at different time points postinjection. It showed the increased accumulation follow by the time course and reaches the maximal at 24 h p.i. The different between tumour(+)/blood and tumour(-)/blood ratios of 386 and 5.4, respectively. It is support to Haubner’s study that the biokinetic of [131I]FIAU shows biphasic elimination. The results showed the use of the [131I]FIAU have a minor influence to further imaging experiment.

Low non-target tissue uptake of radioactivity and obvious tumor:blood ratios suggest that the NG4TL4-STK tumor could be imaged scintigraphically with [131I]FIAU. Planer gamma camera imaging after intravenous injection of [131I]FIAU clearly reflected the transduced tumor. Besides, mice imaging showed non-specific uptake in the stomach and thyroid. The observation of 131I accumulation in the thyroid and stomach in our study indicates the tracer have deiodination. It is pity that we miss stomach in the biodistribution study. Besides,

biodistribution of other tissues and planar imaging show the stable of radiolabelled FIAU, which is almost resistant to metabolic degradation, avoiding radioactivity detection in both target and surrounding tissue. We also observed the contrast between HSV1-TK-expressing tumour tissue and control tumor after GCV treatment. The gamma camera images clearly demonstrated that successful imaging is possible after tracer injection.

Recently, PET imaging shows it may be the preferable imaging system for monitoring gene expression due to its high sensitivity and quantitative character [2, 31, 32]. Further studies, we try to compare the different radiolabelled compounds ([<sup>131</sup>I]FIAU and [<sup>18</sup>F]FHBG) in different original cancer cells by SPECT and PET imaging system to demonstrate the sensitivity, dynamic range and background levels of radioactivity in choosing a reporter probe and in monitoring gene expression. Besides, using human cancer cell lines transduced HSV-tk combined xenograft murine models to examine a potential nuclear imaging in human gene therapy will soon follow.

In conclusion, a tributyltin precursor could be prepared using a simple synthetic strategy. [<sup>131</sup>I]FIAU could be synthesized from this precursor in excellent radiochemical yields. As expected, FIAU was more stable and showed high specific uptake in HSV-tk gene expressing tumor and faster clearance from normal tissues. The optimal time points range between 2 and 4 h. The results support the the FIAU is an excellent agent for transduced HSV-tk gene expression efficiently and selectively, as well as duration of gene expression in vivo in clinical trials.

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