Evaluation of localized bacterial infection using radioisotope-labeled nucleosides imaging modality

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1. Introduction

Conventional diagnostic methods for infections are difficult to distinguish localized bacterial infections from sites of sterile inflammation. For this reason, the importance of developing methods to image bacterial infections is widely recognized [1, 2]. In this study to acquire bacterial infection imaging with radiolabeled nucleosides, in vitro bacterial thymidine kinase (tk) activities of *Salmonella typhimurium* with [^{18}F]FLT and [^{125}I]IVDU were measured and localized infections model in BALB/c mice was imaged with [^{18}F]FLT or [^{125}I]FIAU.

2. Methods and Results

Two different kinds of attenuated *Salmonella* strains, VNP20009 (*msbB*-, *purI*-) and BH129 (*ptsI*-), were genetically engineered to express luciferase (*lux*) genes, and the resulting strains were named as VNP20009-Lux and BH129-Lux, respectively.

2.1 Establishment of bacterial tk by PCR analysis

The PCR reaction mix was prepared by combining 50 pmole of each primer (forward, 5'-AGG TTA GAT ATT CCA GTA CTT TGC-3', and reverse, 5'-ATT AAA CGT AGC GTT CAT TCC CGC-3'). The samples were placed in the PCR machine, the following cycles were run: 95 ℃ for 15 min; 35 cycles of 94 ℃ for 30 sec, 60 ℃ for 1 min, and 72 ℃ for 1 min; and 1 cycle of 72 ℃ for 10 min. The PCR products were then resolved on 2% agarose gel.

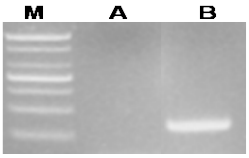


Fig1. These set of primer were used for amplification of a 200bp segment of a bacterial tk gene, respectively.
Lane M : 100bp ladder Lane A : negative control
Lane B : BH129-Lux strain

2.2 [^{18}F]FLT & [^{125}I]IVDU cellular uptake

Bacterial cells were plated at 1×10^4, 5×10^4, 1×10^5 or 1×10^6 colony forming unit (cfu) of two strains in 6-well plate. We determined cellular uptake with 370 KBq of [^{18}F]FLT or 74 KBq of [^{125}I]IVDU according to time intervals at 1×10^6 cfu.

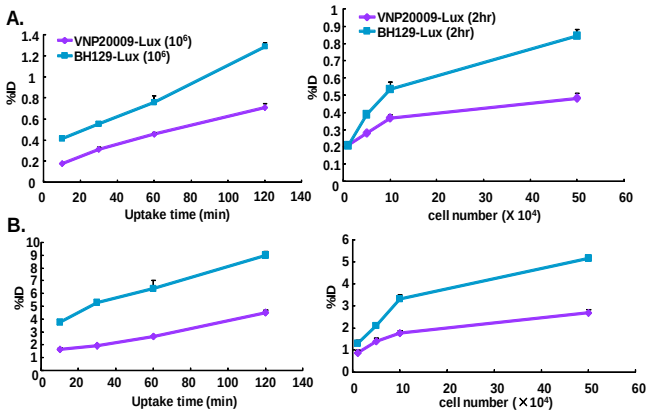
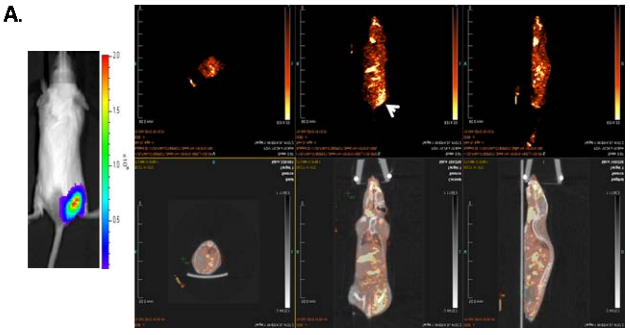


Fig2. In vitro cellular uptake with A. [^{18}F]FLT B. [^{125}I]IVDU. The accumulated radioactivity in two bacterial strains showed a linearly increased pattern with increasing incubation time. In vitro cellular uptake of [^{18}F]FLT or [^{125}I]IVDU were higher in BH129-Lux strain than in VNP20009-Lux strain. [^{125}I]FIAU and [^{18}F]FLT uptake ratio of BH129-Lux to VNP20009-Lux was 2.27, 2.75, 2.41, 1.99 and 2.34, 1.79, 1.66, 1.82 for 10, 30, 60 and 120 min incubation time, respectively.

2.3 In vivo imaging of bacteria infections

Salmonella typhimurium BH129-Lux were used to experimental infections. Localized infections were generated by injecting 1×10^9 into mouse thigh muscles. Four hours later, examinations of the infectious lesions in the thigh muscles of mice were determined by bioluminescence image via lux gene expression. Two hours later, mice were intravenously injected by 0.74 MBq of 11.1 MBq of [^{125}I]FIAU or 7.4 MBq of [^{18}F]FLT and images were taken.



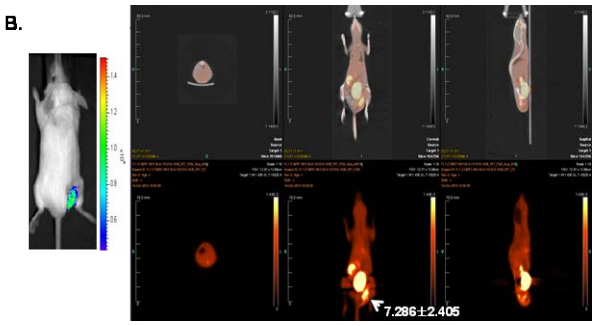


Fig3. In vivo imaging of localized infections with BH129-Lux. After four hours of infection, bioluminescence images were acquired (left panel). Six hours later, bacterial thymidine kinase were confirmed by SPECT/CT (A; [^{125}I]FIAU) and PET/CT (B; [^{18}F]FLT) image. The image clearly demonstrates high uptake of FIAU and FLT in bacterial infection site (right thigh, indicated by arrow). FIAU and FLT uptake in the infection site was mean 7.286±2.405, while the uptake in non-infected site 0.519±0.561.

2.4 Biodistribution

Biodistribution studies were performed on mice injected with 1×10^9 CFU of BH129-Lux into the right thigh. Five hours later, the mice (n=4) were injected with 74 kBq of [^{125}I]FIAU, and sacrificed. Their organs were harvested, and the radioactivity was measured using a gamma counter (1480 WIZARD).The radioactivity of the tissues is expressed as the percentage of injected radioactivity dose per gram of tissue (%ID/g).

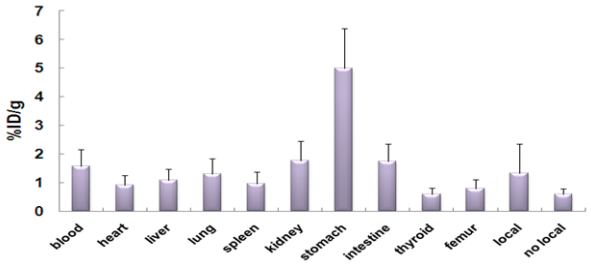


Fig4. The right thigh of each of four mice was inoculated with BH129-Lux (10^9 CFU). Five hours later, mice were injected i.v with 2 μCi of [^{125}I]FIAU. Mice were killed 1 h later, and radioactivity was measured in the indicated organs. The relative activity ratio of localized region to no localized region was 2.98, higher accumulation of [^{125}I]FIAU was shown in localized region.

2.5 Autoradiography

Autoradiography studies were performed in mice injected with 1×10^9CFU of BH129-Lux into the right thigh. Five hours later, the mice were injected with 74 kBq of [^{125}I]FIAU. One hour later, the mice were sacrificed, frozen with tissue freezing medium (FSC22, LEICA, USA) and prepared for sectioning with cryostat microtome (CM1800; LEICA, Germany). The sections were obtained with a thickness of 20 ㎛. The exposure time was 24 hrs. All digital autoradiographic systems were scanned on a BAS-5000 (Fuji Film Co., Tokyo, Japan). The photo-stimulated luminescence (PSL)/mm^2 in the localized infectious region were obtained using software (Bas version 3.0, Fuji Film). The region of interest (ROI) was drawn on the images of the localized infectious region by directly superimposing the digitized photographs of the section.

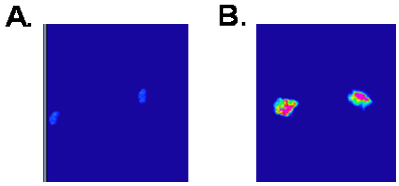


Fig5. Autoradiography showed that the [^{125}I]FIAU uptake in the infected lesion was higher than that in uninfected lesion (Figure 4B).

3. Conclusions

We demonstrated that bacterial thymidine kinase activity was confirmed by cellular uptake of [^{125}I]IVDU or [^{18}F]FLT and bacterial infection in mice also imaged by nuclear medicine imaging with [^{125}I]FIAU or [^{18}F]FLT. Therefore, the localized bacterial infection in living mice could be monitored using a radiolabeled bacterial tk substrate with nuclear medicine modality.

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