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Apoptosis imaging with Iodine-124 labeled Annexin V in Fas-mediated hepatic apoptosis model

Tae Sup Lee [a], Kwang Sun Woo [a], Wee Sup Chung [a], Kyung Min Kim, Jae Hong Kim [b], Kwon Soo Chun [b], Chang Woon Choi, Sang Moo Lim, Gi Jeong Cheon.
[a] *Lab. of Nuclear Medicine,* [b] *Lab. of Radiophamaceuticals Korea Institute of Radiological and Medical Sciences (KIRAMS), 215-4 Gongneung-Dong, Nowon-Gu, Seoul, 139-706, nobelcow@kcch.re.kr*

1. Introduction

Healthy cells and, to a lesser extent, malignant cells undergo apoptosis or programmed cell death in response to a variety of stimuli. At an early stage in this process the cell membrane changes so that phosphatidylserine (PS), a lipid normally present on the membrane's inner surface, is exposed on the outer surface [1]. This change in the membrane can be detected by the binding of annexin V to the external PS, and this has formed the basis for an in vitro assay for apoptosis.

Blankenberg et al. [2] have applied annexin V to the in vivo imaging of apoptosis by labeling annexin V with 99mTc. With this technique, they have been able to image apoptosis.

To extend the use of annexin V to PET, it would be very desirable to iodinate the molecule. The relatively long half-life (4.2 d) of the positron emitting iodine-124 presents several advantages. For example in vivo detection and quantification of longer term biological processes is possible. Also, this cyclotron-generated radionuclide can be prepared well in advance and the established radioiodine labeling techniques can be applied. However, there are some disadvantages such as a relatively low ratio of disintegrations resulting in positrons (23%) and a rather complex decay scheme resulting in several high-energy gamma emissions (0.6-1.69 MeV). Despite this fact, iodine-124 is still considered to be suitable for positron emission tomography (PET) [3]

In this study, we are investigating the feasibility of apoptosis imaging using iodine-124 labeled annexin V in Fas-mediated hepatic apoptosis model.

2. Methods and Results

2.1 Production of iodine-124

Iodine-124 was produced by proton irradiation (MC50 cyclotron at KIRAMS, Republic of Korea) of enriched ^{125}Te targets [^{125}Te(p,2n)^{124}I] at a rate of 37-74 MBq/Ah (22 MeV). The typical batch yield of ^{124}I was 2.8 mCi/uAh and an isotopic impurity of the less then 1% of ^{123}I at the end of beam (EOB) was determined. The radioactivity was extracted by dry distillation and trapped as [^{124}I]NaI in 0.01M NaOH. pH 7.0.

2.2 Radiolabeling of Annexin V with iodine-124

In a eppendorf vial sodium ^{124}I iodide (7.4 MBq; 0.04 ml) was premixed with sodium phosphate buffer (500 mM, pH 7.5, 0.2 ml) followed by addition of Iodobead (Pierce, co) for 10 min and then Annexin-V (Sigma; 1 mg/ml, 0.02ml) in sodium phosphate buffer (50 mM; pH 7.5) was added to reaction vial and incubated 60 min. ^{124}I-labeled annexin V was purified by microcon YM-10 (Milipore, co). The fractions of incorporated ^{124}I were measured by ITLC-sg. A more than 90% labeling yield and 98% radiochemical purity were obtained (Fig 1).

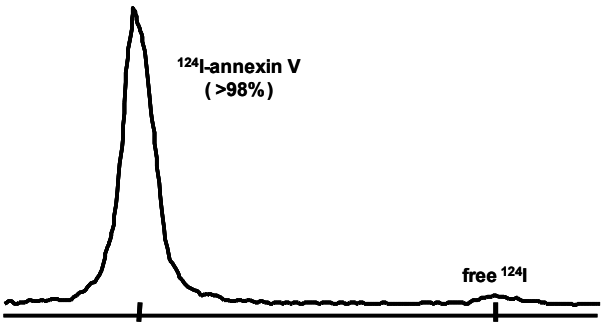

Figure 1. TLC radiochromatogram of purified ^{124}I-annexin V.

2.3 Biodistribution and MicroPET imaging of ^{124}I-annexin V

In order to determine whether the iodine-124-labeled proteins localized to apoptotic lesions in vivo, 6- to 8-week-old female Balb/c mice treated with anti-Fas antibody (clone Jo2, Pharmingen) (to induce apoptosis of hepatocytes). Ninty min after administration of anti-Fas antibody by intravenous injection, Balb/c mice were injected intravenously with iodine-124-labeled annexin V (148 KBq per mouse for biodistribution mice and 1.48 MBq per mouse for imaging studies).

Blockade of specific uptake of free iodide was achieved by intraperitoneal injection of sodium percholate (10 mg/ Kg) 90 min before radiotracer injection. mice were either imaged or sacrificed for biodistribution analysis.

For biodistribution experiments, animals were sacrificed 1 h after radiotracer administration. Organs were excised, weighed and assayed for ^{124}I radioactivity in a gamma counter to determine tissue uptake as a percentage of the injected radioactivity dose per gram of

tissue (% ID/g). A minimum of four animals per treatment group were used for biodistribution analysis.

Immediately prior to imaging, the mice were anesthetized for the data acquisition period (approximately 60 min). Anesthesia during imaging was maintained with 1.5–2% isoflurane in oxygen administered by inhalation (2 l/min).

Mice were placed in a spread prone position and injected with 400 uCi radiolabeled ^{124}I-annexin V into the tail vein in a volume of 0.1 ml. Imaging was carried out on microPET-R4 (Concorde Microsystems Inc., Knoxville, TN) and acqusition time was 60 min. The microPET images were reconstructed with 2-dimensional ordered subset expectation maximization (OSEM) algorithm, after rebinning using Fourier rebining (FORE).

Biodistribution data from apoptosis-negative animals injected with iodine-124 labeled annexin V demonstrate low uptake in all tissues except stomach, intestine ad thyroid.. Treatment with anti-Fas antibody 90 min prior to radiotracer injection resulted in apoptosis positive mice These mice had a higher hepatic accumulation of radiotracers compared with untreated control mice ($P<0.01$). (Fig.2).

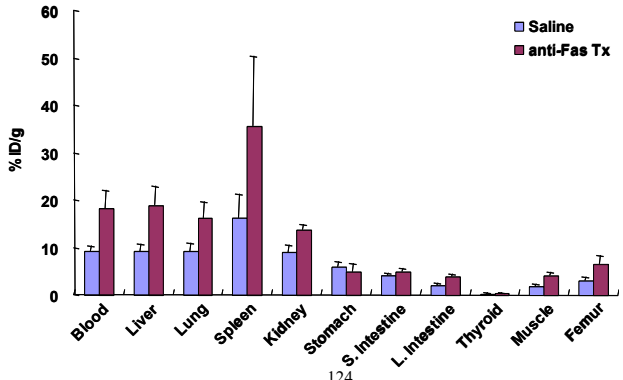

Figure 2. Biodistribution of ^{124}I-Annexin V in saline-treated (control) and anti-Fas antibody treated mice at 1 h post-injection.

In in vivo PET imaging using ^{124}I–annexin V, ^{124}I-annexin V was selectiviely localized in Fas mediated apoptotic liver in vivo and showed low uptake in normal liver of saline treated mice (Fig. 3).

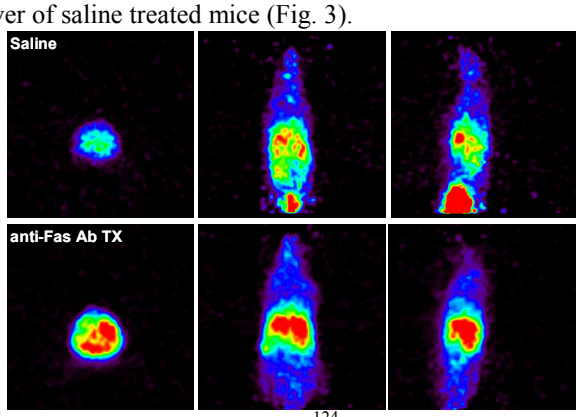

Figure 3. MicroPET image of ^{124}I-annexin V in saline-treated (control, upper panel) and anti-Fas antibody treated mice (lower panel) at 1 h post-injection. transverse (Left), coronal (center) and saggital (right) images were presented.

3. Conclusion

In conclusion, in vivo PET imaging using ^{124}I–annexin V demonstrating that ^{124}I-annexin V could be used to detect regions of cell death in vivo.

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