*Transactions of the Korean Nuclear Society Autumn Meeting*
*Gyeongju, Korea, November 2-3, 2006*

# Apoptosis imaging using dual modality with I-124 annexin V and DEVD-aminoluciferin

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## 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. [3]

Fas (CD95/Apo1) is a 43-kDa cell surface glycoprotein belonging to the tumor necrosis factor receptor family. Ligation of the Fas receptor induces apoptosis by promoting proteolytic cleavage of intracellular caspases. *In vivo*, Fas mediates apoptosis in numerous tissues; the liver seems especially sensitive, because systemic administration of agonistic anti-Fas antibody to mice causes massive death of hepatocytes. Animals treated with anti-Fas die within a few hours, with evidence of gross hemorrhage into the liver parenchyma [4]

Bioluminescence imaging has emerged as a useful and complementary experimental imaging technique for small animals. Bioluminescence with luciferases from firefly and d-luciferin, have been used to monitor two or more biological processes in a single animal. At the molecular level, the members of a family of cysteine proteases (caspases) have been shown to be involved in Fas-mediated programmed cell death. The substrate-binding cleft of caspase-3, the main effector caspase in mammalian cells, recognizes a short 4-aminoacid stretch (DEVD) within protein substrates, directly Nterminal to the cleavage site [5]. This tetrapeptide motif, which is sufficient to bind specifically to the active caspase, has been the basis for the design of inhibitors and molecular markers. As caspase-3 plays a vital role in mediating the initiation and propagation of the apoptotic cascade, the ability to image its activation noninvasively will provide a window of opportunity to evaluate therapeutics in live animals.

In this study we evaluated noninvasive apoptosis imaging against caspase-3 activation and phosphatidylserine externalization in Fas mediated hepatic apoptosis using DEVD-aminoluciferin and $^{124}$I-labeled Annexin V, respectively.

## 2. Methods and Results

### *2.1 Radiolabeling of Annexin V with iodine-124*

In a eppendorf vial sodium $^{124}$I iodide (7.4 MBq; 0.04 ml; MC50 cyclotron at KIRAMS, Republic of Korea) 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.

### *2.2 Preparation of Fas-mediated hepatic apoptosis model*

Ad5-Luc(1X10$^9$ PFU) was injected Balb/c mice through tail vein. After 3 days, luciferase expression using Xenogen IVIS-200 was evaluated by D-luciferin injection. For Fas mediated hepatic apoptosis model, Anti-Jo2 (mouse Fas specific) antibody injected into tail vein of liver specific Luc expressing mice.

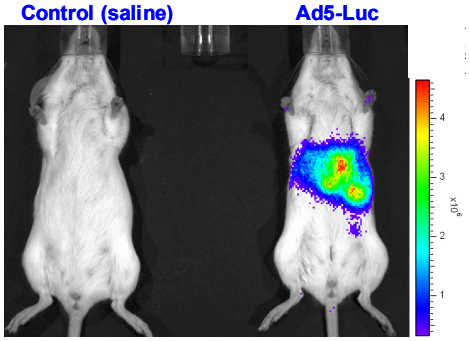

Figure 1. Bioluminescence imaging in liver specific Ad-5-Luc gene delivery model. After Ad5-Luc injection, liver specific luciferase activity was obtained (Fig. 1).

*2.3 MicroPET imaging and Biodistribution*

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). 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. 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.

*2.4 Bioluminescence imaging and Biodistribution*DEVD-aminoluciferin (Promega) i.p injected into liver specific Luc expressing mice and imaged caspase-3 dependent luciferase activity. After imaging, mice was sacrificed, each organs were excised and imaged for the evaluaton of liver specific luminescence.

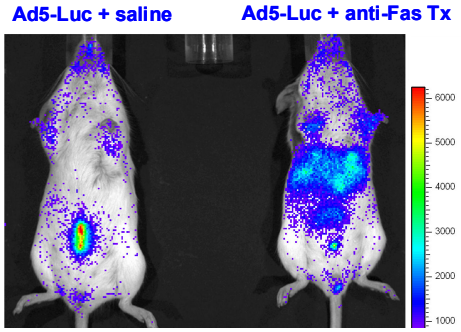


Figure 2. Bioluminescence image of DEVD-aminoluciferin in Fas-mediated hepatic apoptosis of liver specific Luc expressing mice.

Anti-Fas Ab treated mice showed selective uptake of 124I-annexin V in liver (Fig.4) and caspase-3 dependent luciferase activity in liver by DEVD-aminoluciferin (Fig.2 and 4).

$^{124}$I-annexin V uptake in liver of Fas treated mice was 2.06 times higher than in normal mice. Caspase-3 dependent luciferase activity in liver of anti-Fas Ab treated mice was 49.6 fold higher than blood (Fig.3).

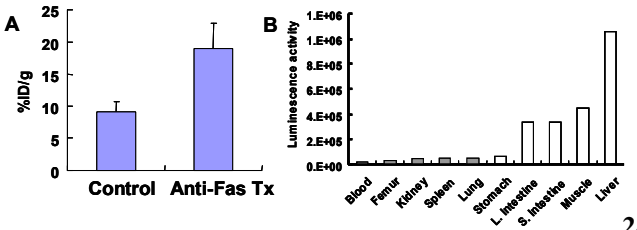


Figure 3. Biodistribution data of (A) $^{124}$I-annexin in Liver and (B) luminescence activity in Fas mediated hepatic apoptosis of liver specific Luc expressing mice.

*2.5 Digital Whole-body autoradiography (DWBA)*For identifying localizaton of apoptosis, MicroPET image and bioluminescence image with the frozen section image obtained by whole body cryotome (Autocryotome, Nakagawa Seisakusho, Japan) in Fas-mediated hepatic apoptosis (Fig. 4).

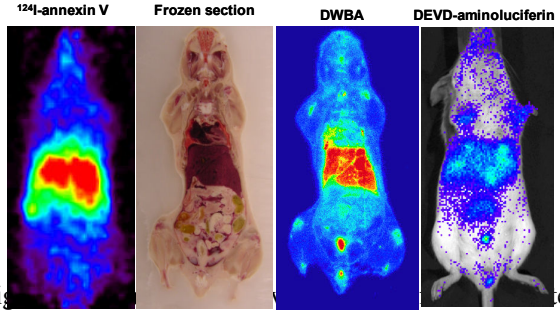


Fig ed hepatic apoptosis of liver specific Luc expressing mice.

## 3. Conclusion

Dual non-invasive imaging, bioluminescence and PET imaging for apoptosis using DEVD-aminoluciferin and $^{124}$I-Annexin V could be useful for the evaluation of apoptosis in cancer therapy monitoring and new drug development.

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