April 11. 2003 (Friday) 12:20~12:50

방사성동위원소로 표지된 단백질을 이용한 방사성의약품의 합성과 평가

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Synthesis and Evaluation of Radiopharmaceutics Labeled with Radioisotope

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◎ 방사성의약품

방사성동위원소를 표지하여 인체에 투여함으로써 질병의 진단 또는 치료에 사용하는 물질.

◎ 방사성의약품의 분류

- 진단용: Gamma ray, Positron ray
  Half life : min ~ hours
- 치료용: Alpha ray, Beta ray
  Half life : over 10 hours ~ scores of days

방사성의약품 사용 국내병원: 약 120개 (2002년)

◎ 방사성의약품의 조건

의약품과 방사성 물질

- 의약품으로서는
  ① 검사 또는 치료의 목적에 적합한 물질
  ② 독성이 없고 장애를 일으키지 않고
  ③ 현재 임상에 보급되어 있는 약품

- 방사성 물질로서의 성질은
  ① 짧은 유효반감기
  ② 적절한 방출 에너지
  ③ 높은 specific activity
  ④ 경제적

◎ 방사성동위원소의 생산

- Nuclear reactor
  131I ~ 166Ho
- Cyclotron
  201Tl, 123I, 67Ga, 111In – gamma ray
  18F, 11C, 13N, 15O - positron
- Generator
  99mTc, 188Re

◎ ^{99m}Tc

^{99m}Tc : 원자번호-43, VIIB족 금속전이원소
여러 가지 ligand와 착체를 잘 형성
Half life : 6 hr

$^{99m}TcO_4^-$: 산화수 +7,
매우 안정하여 ligand의 결합이 어려움.
환원제 : 산화수를 낮추어 줌. (+3, +4, +5)

◎ Tc-CO : Oxidation state +1

Problem in lower oxidation states :
- synthesis was perceived to be difficult
- unstable against hydrolysis and oxidation.

Technetium tricarbonyl ion (+1) :
stable compound

Application of Cancer Diagnosis & Therapy Using ^{99m}Tc-CO-labeled Annexin V

Annexin V

- endogenous human protein (36 kDa)

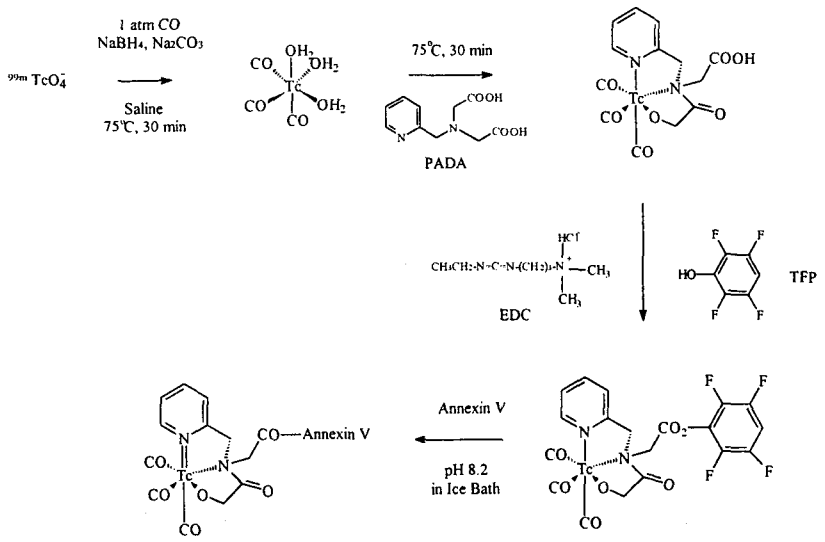
- binding to phosphatidyl serine on the outer leaflet of the cell membranes during apoptosis

- as a marker of cell death using radiolabeled and fluorescence analysis *in vitro* and *in vivo*

Purpose

- To improve the hepatic and renal clearance of ^{99m}Tc labeled pharmaceutical agents

- Annexin V was conjugated with a performed chelate, the TFP ester of [$^{99m}Tc(CO)_3$Picolylamine-*N,N*-diacetic acid] in which the pyridine nitrogen, the tertiary amine and one carboxylic acid to improved clearance properties



Synthesis of $^{99m}Tc(CO)_3$ PADA-Annexin V

Purification & Confirmation

1. **RP-HPLC system**
   water base : 0.05M triethylammonium phosphate
   organic base : Acetonitrile

2. **The conjugate was purified on a PD-10 column**

3. **The purity was confirmed by HPLC using TSK 2000 size exclusion column**

Biodistribution Study

- Test materials : $^{99m}Tc(CO)_3$ PADA labeled annexin V
  Specific activity (5 μCi/ug)
- Animal species: Balb/c mice (5 animals/group)
- Injection route : Tail vein (5 μCi/animal)
- Organs : Liver, kidney, Intestine, Stomach, Spleen, Lung, Bone, Muscle
- Blood preparation: heart puncture
- Radioactivity counting: *r*-counter

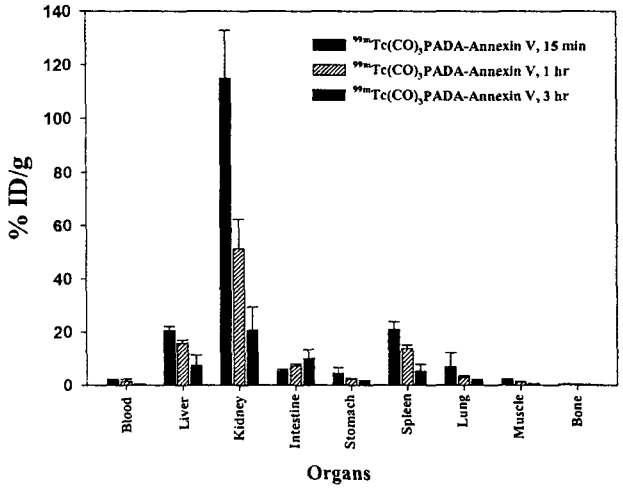


Fig. 1. Biodistribution of $^{99m}Tc(CO)_3$ PADA labeled annexin V in normal mice (n=5).

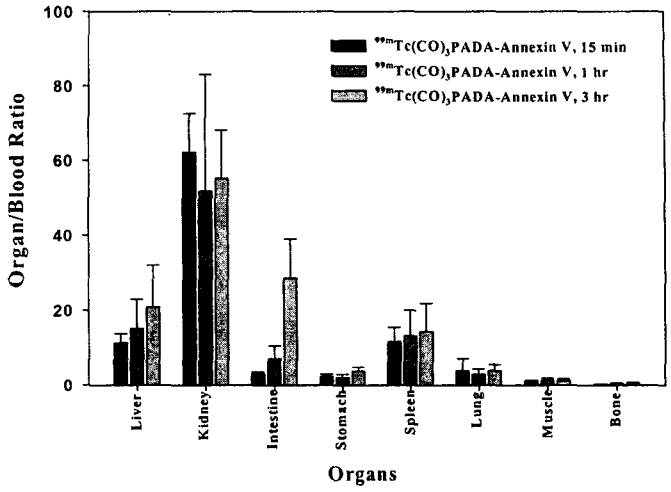


Fig. 2. Organ/blood ratio of $^{99m}Tc(CO)_3$ PADA labeled annexin V in normal mice (n=5).

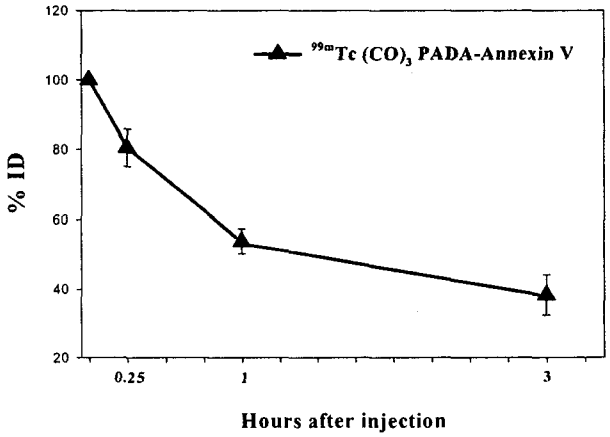


Fig. 3. Whole body radioactivity retention of $^{99m}Tc\ (CO)_3$ PADA labeled annexin V in normal mice (n=5).

Conclusions

- Improved clearance from the major organs was achieved by labeling with a [^{99m}Tc(CO)$_3$ (PADA)] chelate that is excreted readily.

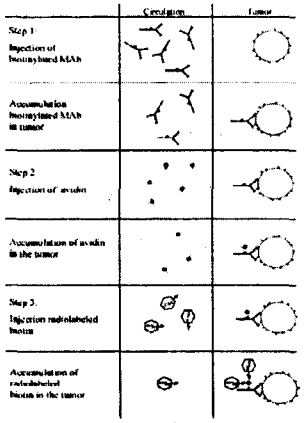
- This [^{99m}Tc(CO)$_3$ (PADA)]-AV warrants further investigation for targeting apoptosis.

Pretargeting Application of Cancer Diagnosis & Therapy Using Radiolabeled Biotin

Problems in Tumor Targeting by Radiolabeled Antibody

- Conventional mAb targeting using directly radiolabeled mAbs is characterized by slow accumulate in tumor.
- The half-life of radiolabeled mAb is circulated for 2-4 days.
- Relatively high residence times of the radiolabeled mAbs in the nontarget tissues
- For these reasons, more effective targeting of tumors with mAbs is required.

Pretargeted Radioimmunotherapy of Cancer



Schematic representation of 3-step avidin/biotin-based pretargeting

Properties of the radiolabels used in pretargeting

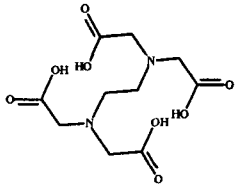
- rapid diffusion into extracellular space
- rapid hepatic and renal excretion
- high specific activity
- hydrophilicity
- no protein binding in the blood
- nonimmunogenicity
- linking agents conjugated with radionuclide are relatively small molecules
- to maximize accumulation in the tumor while minimizing exposure to the nontargeting organs

Purpose

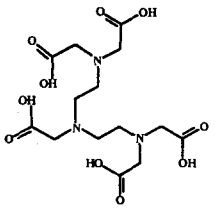
- For imaging using pre-targeted mAb-avidin or -streptavidin, it would be desirable to synthesize a ^{99m}Tc labeled biotin that clears rapidly from the whole-body

- To optimize reagents for potential *in vivo* application, antibody-based pre-targeting of cancer, we have synthesized a number of linking agent conjugated with biotin

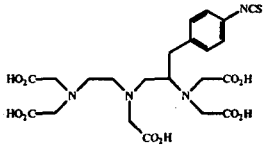
Various linking agents used for pretargeting radionuclide



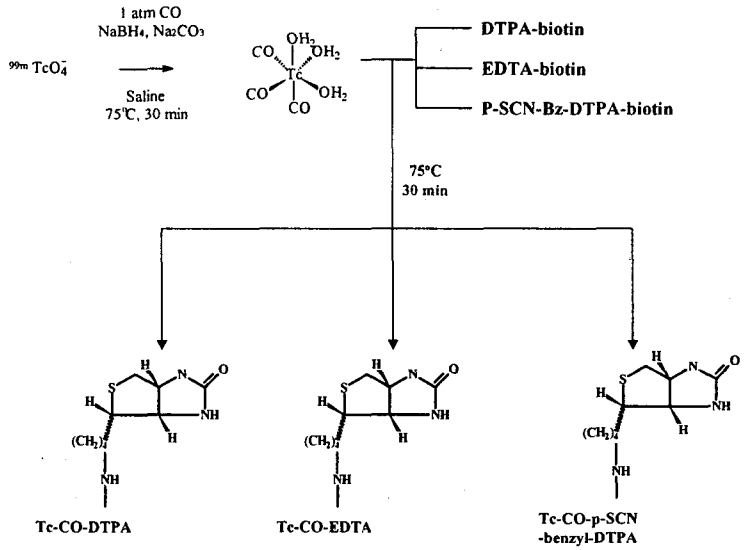
Ethylenediaminetetraacetic acid



Diethylenetriaminepentaacetic acid



p-SCN-benzyl-DTPA



Synthesis of $^{99m}Tc(CO)_3$-DTPA, -EDTA, -p-SCN-Bz-DTPA-biotin

Purification & Confirmation

1. **RP-HPLC system**
   water base : 0.05M triethylammonium phosphate
   organic base : Acetonitrile

2. **The conjugate was purified on a monomeric avidin-gel column**

3. **The purity was confirmed by RP-HPLC**

HPLC retention time of $^{99m}Tc(CO)_3$ labeled various linking agents conjugated with biotin

Biotin derivatives	Retention time
^{99m}Tc-CO-DTPA-Bt	13.31min
^{99m}Tc-CO-EDTA-Bt	13.75 min
^{99m}Tc-CO-*p*-SCN-benzyl-DTPA-Bt	11.23 min

Chemical stability of ^{99m}Tc-labeled biotin derivatives challenged with histidine

Biotin derivatives	2 hr	4 hr	6 hr	12 hr
^{99m}Tc-CO-DTPA-Bt	100%	100%	98.1%	93.7%
^{99m}Tc-CO-EDTA-Bt	95.1%	93.4%	92.5%	86.8%
^{99m}Tc-CO-*p*-SCN-benzyl-DTPA-Bt	100%	98.1%	93.4%	87.0%

Biodistribution Study

- **Test materials : ^{99m}Tc(CO)$_3$ DTPA-, EDTA-, p-SCN-benzyl-DTPA-labeled Biotin**
  (Specific activity, approximately 5 μCi/ug)
- **Animal species: Balb/c mice (5 animals/group)**
- **Injection route : Tail vein (5 μCi/animal)**
- **Organs : Liver, kidney, intestine, stomach, spleen, lung, bone, muscle**
- **Blood preparation: hart puncture**
- **Radioactivity counting: *r*-counter**

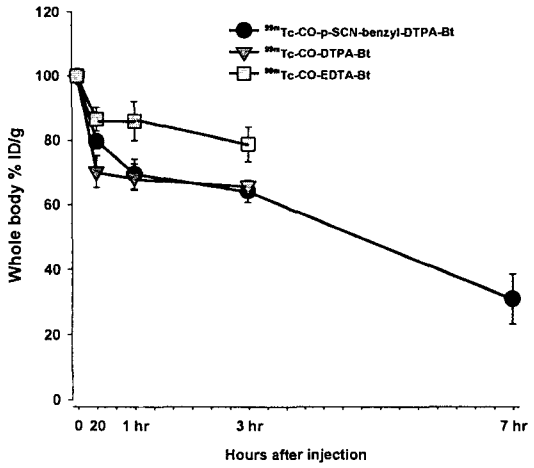


Fig. 4. Whole body radioactivity retention (% ID) of ^{99m}Tc-CO-labeled biotin after i.v. injection in normal balb/c mice

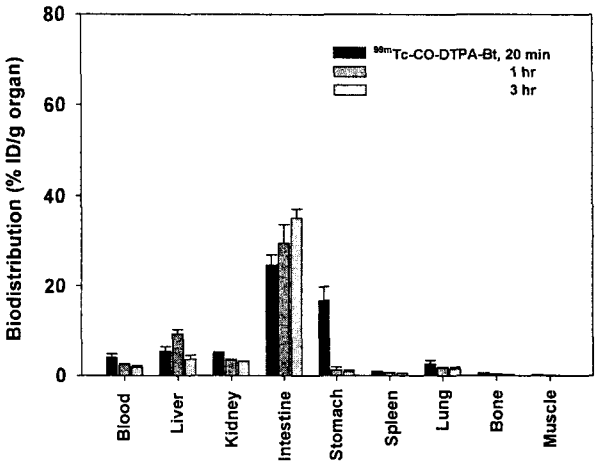


Fig. 5. Biodistribution of ^{99m}Tc(CO)$_3$-DTPA-biotin after i.v. injection in normal balb/c mice

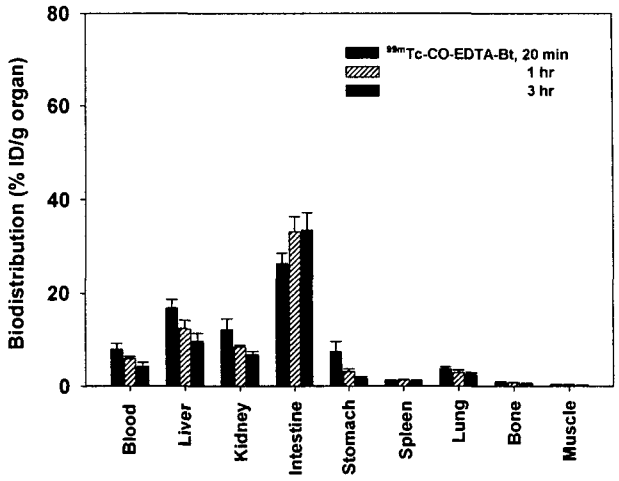


Fig. 5. Biodistribution of $^{99m}Tc(CO)_3$-EDTA-biotin after i.v. injection in *normal balb/c mice*

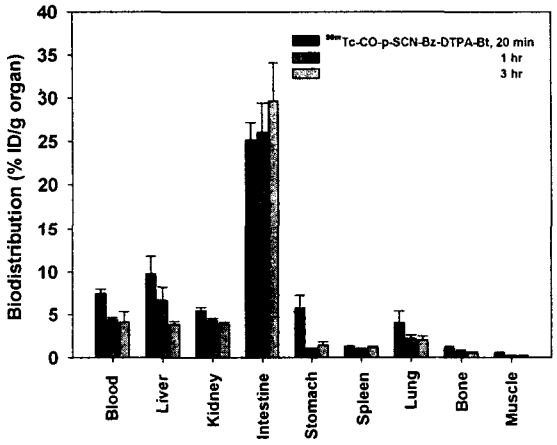


Fig. 6. Biodistribution of $^{99m}Tc(CO)_3$-p-SCN-Bz-DTPA-biotin after i.v. injection in normal balb/c mice

Conclusions

- **This study showed an inverse relationship between the whole body retention and the polarity of the labeled biotin.**
- **A chemical insertion of more polar groups is needed to make ^{99m}Tc $[(CO)_3$ (DTPA-Bt)] clear more rapidly via the renal system.**