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^{123}I, ^{99m}Tc, ^{111}In 표지 사람비특이 항체와 ^{111}In Oxine 표지 백혈구의 포도상구균 농양유발 백서에서의 동태비교

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임상무 • 전권수 • 우광선 • 정위섭 • 이종두

= 국문초록 =

Comparison Polyclonal IgGs Labeled with ^{123}I, ^{99m}Tc, ^{111}In and ^{111}In Oxine Leukocytes in the *Staphylococcal* Abscess Bearing Rats

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감염병소의 진단을 위해 여러 방사성성핵종 표지 사람비특이항체들이 임상이용되었으나, ^{123}I, ^{99m}Tc, ^{111}In등 표지 핵종과 표지방법에 따른 체내동태의 차이에 대한 자료가 필요하며, 감염병소의 진단에 표준적으로 이용되어지던 ^{111}In-oxine 표지백혈구와 비교평가도 요구된다. 저자들은 10^9개의 포도상구균을 좌측 대퇴부에 주사하여 농양을 유발한 백서에서 ^{123}I 표지, iminothiolane을 이용한 ^{99m}Tc 표지, DTPA이용 ^{111}In 표지 사람비특이항체와 ^{111}In-oxine 표지 백혈구의 체내동태 및 농양 섭취율을 비교하였다. ^{123}I-IgG는 갑상선 및 위의 방사능이 높아 체내 탈요드반응이 빠름이 시사되었으며, ^{99m}Tc-iminothiolane IgG는 신장방사능이 높아 신장으로 IgG 또는 대사물이 배설됨을 알 수 있었다. ^{111}In-oxine표지 백혈구는 간 및 비장의 방사능이 높았고, 혈액방사능 제거율이 가장 빨랐다. 주사 24시간 후의 농양섭취율은 ^{111}In-DTPA IgG가 가장 높았고, 농양 대 혈액 방사능비는 ^{111}In-oxine표지 백혈구가 가장 높았으며, ^{111}In-DTPA IgG와 ^{99m}Tc-iminothiolane IgG가 다음으로 비슷하였다. ^{111}In-oxine표지 백혈구보다는 방사성핵종표지 IgG가 간편하게 이용될 수 있으며, ^{111}In이 ^{99m}Tc나 ^{123}I보다 지연영상의 촬영에 유리함을 알 수 있었다.

Key Words : Human Polyclonal IgG(HIG), Abscess, ^{123}I, ^{99m}Tc, ^{111}In

INTRODUCTION

Several radionuclide imaging techniques have been developed for the detection of infection and inflammation[1]. ^{67}Ga-citrate and ^{111}In oxine labeled leukocytes were the principle scintigraphic agents employed clinically for imaging infection. However the normal route of excretion of ^{67}Ga into the bowel lumen compromises its sensitivity and specificity for the detection of abdominal infection foci. ^{111}In oxine labeled leukocytes require a prolonged and complicated laboratory preparation procedure, and a small administered dose

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Table 1. Biodistribution of Radiopharmaceuticals at 4 hrs after Injection in the Abscess Bearing Rats(7 Heads for each Group)

	^{123}I-IgG		^{99m}Tc-IgG (2-iminothiolane)		^{111}In-IgG (DTPA)		^{111}In-WBC (oxine)	
Organs	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Blood	1.91	0.11	2.33	0.39	2.76	0.11	1.39	0.21
Liver	0.94	0.13	1.44	0.34	1.60	0.22	7.29	1.30
Spleen	0.64	0.16	1.08	0.13	1.44	0.30	20.11	5.71
Kidney	0.78	0.11	8.08	0.87	1.95	0.29	1.28	0.25
Sternum	0.27	0.08	0.28	0.05	0.35	0.05	0.55	0.21
Femur	0.32	0.06	0.35	0.05	0.51	0.06	0.80	0.28
Muscle	0.16	0.05	0.14	0.02	0.16	0.04	0.11	0.04
Thyroid	0.59	0.29	0.11	0.06	0.43	0.12	0.06	0.02
Lung	0.91	0.15	1.11	0.23	1.48	0.13	2.07	0.35
Stomach	1.31	0.39	0.31	0.02	0.20	0.03	0.19	0.05
Abscess	0.66	0.21	0.80	0.37	1.47*	0.17	0.40	0.14

*$p<0.05$

limits image resolution due to low photon flux.

Recently monoclonal antibodies against leukocytes are developed for *in vivo* leukocytes labeling, but there are doubts about the repeated use of a non-human protein because of possible host reaction against the agent[2]. Radiolabeled human polyclonal immunoglobulin has been shown to have a high sensitivity and specificity for imaging focal sites of inflammation in both animal models and human subjects[3-7]. Immunoscintigraphy with various monoclonal antibodies revealed different biodistribution and metabolism when they were labeled with various radioisotopes and labeling techniques[8-15,26,27]. In this study, we prepared ^{123}I, ^{99m}Tc and ^{111}In labeled human polyclonal IgG and ^{111}In oxine labeled leukocytes[1,14,16,17]. The biodistribution and imaging properties were compared in the *Staphylococcal* abscess bearing rat.

MATERIALS AND METHODS

1. Materials

Cyclic DTPA anhydride, 2-iminothiolane, dimethylsulfoxide and human nonspecific polyclonal IgG were obtained from Sigma (St. Louis MO). $Na^{99m}TcO_4$ were obtained from Korea Atomic Energy Research Institutes. $Na^{123}I$, ^{111}In citrate and ^{111}In oxine were produced by the MC-50 cyclotron in the Korea Cancer Center Hospital. Sprague Dawley rats with body weights around 200 grams were used.

2. Labeling IgG with ^{123}I

100ug of IgG was dissolved in 20ul of 0.01M PBS, and $Na^{123}I$ 300uCi in 0.2M phosphate buffer, pH 7.4 was added. 25ug of Chloramine-T in 10ul water was added to the mixture and incubated at room temperature for 30 minutes. Sodium metabisulfite was added to stop the reaction. Labeled IgG was separated immediately with a 1.6×100cm Sepharose 6LB column.

3. Labeling IgG with ^{99m}Tc using 2-iminothiolane

27.5ug of 2-iminothiolane was added to 10mg of IgG in 1 ml of 0.1M phosphate buffer pH 7.4, and stirred at ambient temperature for 12 hours. The modified IgG was separated from the excess reagent by filtration through a Sepharose 6LB column using 0.1M citrate buffer, pH 6.5. To label glucoheptonate 15mCi of $Na^{99m}TcO_4$ was

Table 2. Biodistribution of Radiopharmaceuticals at 24 hrs after Injection in the Abscess Bearing Rats(7 Heads for each Group)

	^{123}I-IgG		^{99m}Tc-IgG (2-iminothiolane)		^{111}In-IgG (DTPA)		^{111}In-WBC (oxine)	
Organs	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Blood	1.90	0.12	0.66	0.09	1.67	0.16	0.55*	0.08
Liver	0.52	0.08	0.90	0.14	1.37	0.08	10.16	1.15
Spleen	0.40	0.08	0.88	0.27	1.66	0.39	21.04	6.60
Kidney	0.50	0.04	11.76	0.48	2.08	0.20	1.88	0.24
Sternum	0.28	0.04	0.17	0.04	0.41	0.04	0.61	0.23
Femur	0.27	0.02	0.27	0.05	0.56	0.04	0.92	0.29
Muscle	0.23	0.06	0.14	0.03	0.38	0.11	0.21	0.07
Thyroid	3.59	0.47	0.06	0.01	0.25	0.06	0.04	0.01
Lung	0.60	0.05	1.40	0.06	1.01	0.11	1.38	0.51
Stomach	2.26	0.67	0.19	0.02	0.45	0.05	0.20	0.05
Abscess	0.56	0.07	0.72	0.26	1.72*	0.24	0.68	0.20

*$p<0.05$

added to the vial containing 40mg of glucoheptonate and 0.35mg of $SnCl_2$. 150ug of 2-iminothiolane coupled IgG and 15mCi of ^{99m}Tc-glucoheptonate were mixed and left at ambient 6LB column using 0.01M PBS, pH 7.4.

4. Labeling IgG with ^{111}In-citrate

50ug of cyclic DTPA anhydride was dissolved in 70ul of dimethylsulfoxide, and was added to the 10mg of IgG in 1 ml 0.1M PBS. The reaction mixture was left to stand for 1 hour at room temperature. Then unconjugated DTPA was removed from DTPA conjugated IgG by a Sepharose 6LB column (1.6×100cm) eluted with 0.01M PBS, pH 7.4. 3mCi of ^{111}In-citrate was mixed with 150ug of DTPA conjugated IgG was separated by filtration through a Sepharose 6LB column using 0.01M PBS, pH 7.4.

5. Labeling Leukocytes of Rat with ^{111}In-oxine

10ml of blood was withdrawn from the heart of Sprague Dawley rats with heparinized syringes. a same volume of Hanks balanced salt solution was added to the heparinized blood. The mixture was layered on the Ficoll 119, and centrifuged at 400 g for 30 minutes. The leukocyte rich plasma layer was separated, a same volume of HBSS was added, and centrifuged again at 400g, for 15 minutes. The leukocytes pellet was resuspended with 1 ml of 0.9% NaCl, mixed with 1 mCi of ^{111}In-oxine, and left to stand for 30 minutes at room temperature. ^{111}In-oxine labeled leukocytes were washed with 0.9% NaCl.

6. Biodistribution of Radiopharmaceuticals in Rats with Abscess

Staphylococcus aureus were cultrured and one billion cells were injected into the left thigh of Sprague Dawley rats. 24 hours after injection, the biodistribution of radiopharmaceuticals were observed 4 and 24 hours after injection. 6 heads of rats were used for each group.

The data were analyzed statistically using the Student t test or analysis of varience(ANOVA).

RESULT

1. Radiolabeling IgG

The IgG without 2-iminothiolane was labeled directly with ^{99m}Tc glucoheptonate in small amount. Increasing the amount of 2-iminothiolane

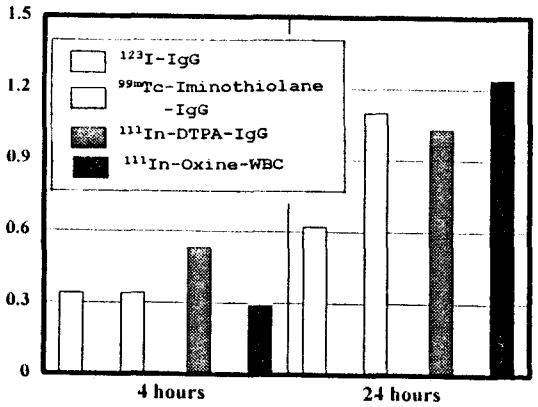

Fig. 1. Abscess to blood radioactivity ratios at 4 and 24 hours after injection of radiopharmaceuticals.

for derivatization IgG improved the labeling yield.

Labeling yied with ^{123}I was 73%, with ^{99m}Tc 56 %, with ^{111}In 68% respectively and after purification with PD-10 column radiochemical purity were above 95%.

2. Distribution of Radiolabeled IgG in the *S. aureus* Abscess Bearing Rats

Blood clearance was fastest with ^{111}In-oxine-WBC($p<0.05$). Change in molar excess of 2-iminothiolane in derivatization of IgG did not alter the biodistribution.

Activities of ^{123}I-IgG in the thyroid and stomach at 24 hours suggest deiodination in the body. Activities in the liver and spleen were highest with ^{111}In-oxine-WBC. Renal activity was highest with ^{99m}Tc-2-iminothiolane-IgG.

Activity in the abscess was highest with ^{111}In-DTPA-IgG($p<0.05$). But abscess/blood ratio was highest with ^{111}In-oxine-WBC(Table 1, 2, Fig. 1).

DISCUSSION

The radiolabeled human nonspecific polyclonal IgG was thought to be bound to the Fc receptor on the surface of phagocytes, but increased blood flow, vascular permeability, binding to bacteria and other factors seem to be important[18,20]. In rats with a calf muscle infection, ^{111}In-IgG, ^{111}In-IgA, ^{111}In-HSA showed similar abscess to background ratio. But the absolute abscess uptake of ^{111}In-IgA was significantly lower, because ^{111}In-IgA accumulated more in liver, spleen and kidney. This suggests that the Fc-r receptor is not crucial for accumulation in infection foci[21].

Biodistribution of radiolabeled IgG can be different with various labeling method and radionuclides. Comparing ^{123}I, ^{99m}Tc, ^{111}In labeled IgG, ^{111}In is reported to have highest abscess uptake. Radioiodine has been used most widely for protein radiolabel, because of the easy direct radioiodination. But once internalized by cells, catabolism release peptide fragments or free amino acids with further metabolic processing ultimately releasing radioiodide. Activities of ^{123}I-IgG in the thyroid and stomach at 24 hours suggested deiodination *in vivo*. The deiodination of IgG may cause lower uptake in the abscess. Another limit of ^{123}I is that it is expensivelt produced from the cyclotron. DTPA conjugated IgG is known to show high hepatic retention especially after labeling with ^{111}In, which is known to be due to its metabolites. Labeling 2-iminothiolane conjugated IgG with ^{99m}Tc-glucoheptonate made mixture of direct labeled and chelate labeled IgG. Tin chloride was used to label glucoheptonate with ^{99m}Tc which can reduce disulfide bond in the IgG. High renal radioactivity of the ^{99m}Tc-iminothiolane IgG suggested renal excretion of the metabolites, and can make it difficult to diagnose the infection around the idney. The combinatin of superior imaging properties, wide spredad availability, and low cost indicate preference for ^{99m}Tc when possible. Since proteins are typically slow to target and slow to disappear from blood, their distribution properties may not be complementary with

the 24 hour ^{99m}Tc window of imageability given its 6-hour half-life. The 2-iminothiolane or similar direct labeling approach depends on achieving a balance of stability and instability for imaging [21]. With other chelates or linker, ^{99m}Tc-IgG may show different imaging properties.

Leukocytes of rats are smaller than human leukocytes, which was not separated with simple sedimentation, and density gradient centrifugation with Ficoll was needed. In that separated leukocytes, lymphocytes were mixed together. The complexity of separating the leukocytes is one major drawback. Uptake in the liver and spleen was highest with ^{111}In leukocytes, which seemed to be the cause of the fastest blood clearance[29]. Although the abscess to blood ratio was best with ^{111}In leukocytes, it may be difficult to detect the abscess around the liver and spleen, which is another drawback.

Staphylococcus aureus can bind IgG to the protein A on its cell wall, and may affect the uptake of the radiolabeled materials. That may cause higher uptake of ^{111}In IgG than ^{111}In leukocytes in the abscess. For the clinical use, ^{111}In IgG seems to be best, because it has suitable half life for the delayed imaging.

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