COMPARISON OF BIODISTRIBUTION OF ^{111}IN-TROPOLONE LEUKOCYTES AND ^{125}I-HUMAN NONSPECIFIC POLYCLONAL IgG IN NORMAL AND INDUCED INFLAMMATION MICE FOR DETECTION OF INFLAMMATION

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ABSTRACT

Human nonspecific polyclonal IgG and granulocytes, which accumulate in inflammation foci, were radiolabeled with ^{125}I and ^{111}In-Tropolone, respectively. Biodistribution of these two radiolabels was assessed in normal and inflammation-induced mice. ^{125}I-IgG showed better localization to the inflammated areas. Blood levels with ^{111}In-Tropolone leukocytes were lower at all time points. In addition, the inflammatory thigh-to-blood ratios showed an improvement, whereas the ratios of inflammatory thigh-to-other normal tissues were higher for ^{125}I-IgG than ^{111}In-Tropolone leukocytes. In conclusion, labeled IgG due to better localization in inflammated sites and higher target-to-background ratios is more suitable agent than labeled leukocytes for immunoscintigraphy of inflammation.

Key words: Immunoscintigraphy, Acid Citrate Dextrose (ACD), Hydroxy Ethyl Starch (HES), Turpentine, Immunoglobulin (Ig), Percent Injected Dose per gram tissue (% ID/g)

INTRODUCTION

Various nuclear medicine techniques are widely used to image foci of infection and inflammation, such as radiolabeled antibodies and granulocytes. Among the relatively new radiopharmaceuticals for this purpose, are radioimmuno-conjugates such as labeled murine monoclonal antigranulocyte antibodies and labeled human nonspecific polyclonal IgG (1).

Although the mechanism for accumulation of radiolabeled nonspecific human IgG in inflammatory and infectious foci is not completely clear, it is likely related to increased vascular permeability into an expanded extracellular fluid space and to the chemical nature of the radiolabel (2). However, the main factor limiting the clinical application of radiolabeled antibodies is an often-moderate target-to-background ratio in image (3). To overcome this, several methods have been proposed. Most of investigations have been focused on improvement radiolabeling methods of antibodies resulting in reduction of radioactivity of normal tissues and enhancement of target-to-background ratio. As much as target-to-background ratio increases, inflammation would be more separable from background in images. The use of leukocytes labeled with ^{111}In to locate inflammation is an established and clinically useful technique. If leukocytes are radiolabeled with a gamma emitting compound, their accumulation (more granulocytes) in the reticuloendothelial system tissues and inflammatory sites can be detected as hot spots on a gamma camera image (4). Due to problems in radiolabeling leukocytes and also accumulation of leukocytes in reticuloendothelial system, investigators have focused on other techniques like antibody scintigraphy for detection of inflammation.

The main objective of this study was to compare immunoscintigraphy of inflammation with antibody and leukocytes; therefore, the biodistribution of IgG (labeled with Na^{125}I by Chloramin-T method) and leukocytes (labeled with ^{111}In-Tropolone) was studied in normal and inflammation bearing mice.

MATERIALS AND METHODS

^{125}I as Na^{125}I with the specific activity of 100 μci/μl in NaOH was purchased from Amersham.

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IgG was prepared from human plasma (5) and was iodinated by Chloramin-T method (6). Briefly, 100 µg of IgG was incubated with 2 mci of $Na^{125}I$ and 25 µg of chloramin-T for 1min. Free radioactive iodine was separated by Sephadex G-50 column chromatography. The specific activity of ^{125}I-IgG was estimated to be between 5-10 mci/mg.

^{111}In as $^{111}InCl_3$ with a specific activity of 30 mci / ml in 0.01 N HCl was obtained from Cyclotrone Division, Agricultural & Nuclear Medicine Research Center, Atomic Energy Organization of Iran, Karaj. Radioactive samples were measured using NaI gamma counter.

Leukocytes (WBC) were labeled as follow (4):

18 ml of venous blood were drawn into 2×25 ml sterile disposable plastic syringes, each containing 2.25 ml ACD. Four-ml HES 6% was added to each syringe. Allowed the syringes to sediment for 45-60 min at room temperature. After sedimentation of supernatants at 450g for 5 min, cell pellet was gently resuspended in 0.5 ml cell free plasma (CFP_1), which was obtained from centrifugation of 5 ml blood at 2000g for 15 min. The platelet rich plasma was centrifuged at 2000g for 15 min to obtain cell free plasma (CFP_2). 70 µl Tropolone at a concentration of 1 mg / ml in HEPES buffer (0.2 M, pH 7.5) was added to 50 µl $^{111}InCl_3$ (about 1500 µci). After 15min incubation at room temperature with gentle swirling, 5 ml of CFP_2 was added and the mixture centrifuged at 450g for 5 min. The cell pellet resuspended in CFP_1 for injection.

An animal model was developed injecting 40 µl of Turpentine in the posterior left thigh of Balb/c mice weighing approximately 25g. Mice were left for 48 hour in normal condition to develop the inflammation foci. 100 µci / 0.1 ml / 20 µg ^{125}I-IgG / mouse or 20 µci / 0.1 ml ^{111}In–Tropolone leukocytes / mouse was injected through tail veins into mice. Six mice from each group were killed with ether for determination of tissue radioactivity at times 2, 4, 6, 24, 30, and 48 hour post administration of radiolabeles. Selected organs (Blood, Spleen, Liver, Stomach, Intestine, Kidney, Left Thigh, Right Thigh, Bone, and Lung) were removed and placed into pre weighed tubes and radioactivity was measured. The percent of injected dose per gram tissue (% ID/g tissue) was determined. The total injected dose was calculated by measuring syringes before and after injection to each animal.

Statistical analysis

All values were expressed as mean ± standard deviation (Mean±SD) and data were compared using student T-test. Statistical significant was defined as $P< 0.05$.

RESULTS

Results of the biodistribution in normal and induced inflammation mice at 2, 4, 6, 24, 30 and 48 hr post injection of ^{125}I-IgG and ^{111}In-Tropolone leukocytes (n=6 per time point) are shown in Figures 1-4.

There was no significant difference between the radioactivity of left and right thigh in mice without inflammation after injection of ^{125}I-IgG and ^{111}In-Tropolone leukocytes (Tables 1,3). In inflammation bearing mice, radioactivity values of left and right thigh were significantly different ($P<0.05$) at definite times (6, 24, 30, and 48hr for labeled IgG, 24 and 30hr for labeled leukocytes) after injection of ^{125}I-IgG and ^{111}In-Tropolone leukocytes (Tables 2,4).

Compared with ^{111}In-Tropolone leukocytes, blood radioactivity of ^{125}I-IgG was much higher, but radioactivity in the other organs, especially in the liver, spleen, and kidney were lower. The maximum radioactivity of normal tissues and inflammatory (left) thigh was achieved 4 hr after injection of ^{125}I-IgG, and reduced dramatically after 4 hour (e.g. % ID/g of blood from 29 at 4 hr to 13 at 6 hr and 7.5 at 24 hr). The reduction of radioactivity in inflammatory (left) thigh was not as much as reduction in normal tissues (% ID/g from 7 at 4 hr to 6 at 6 hr and 5 at 24 hr). The best inflammatory thigh-to-normal tissue ratios were obtained at 24hr post administration of ^{125}I-IgG (Table 5).

Following administration of ^{111}In-Tropolone leukocytes there was high radioactivity in blood, liver, spleen, and lung. Two hours after injection, radioactivity decreased in blood and lung, whereas it increased in liver and spleen. Therefore, blood levels were lower at all times; consequently, inflammatory thigh-to-blood ratios were improved. But as is apparent from Table 5, inflammatory thigh-to-liver, spleen, and kidney ratios were low and the best ratios were obtained at 48 hr post administration of ^{111}In-Tropolone leukocytes.

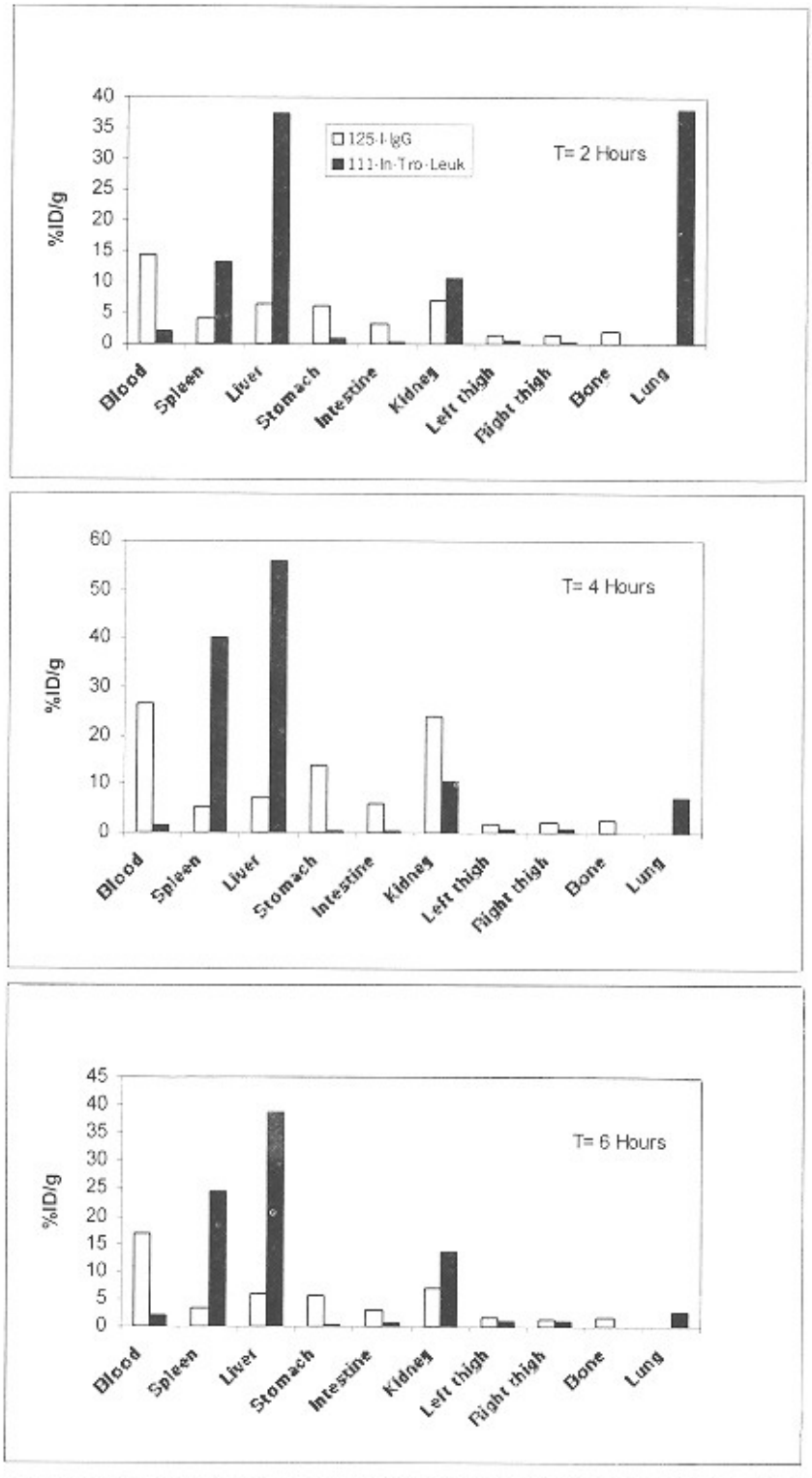

Fig 1. Histograms showing biodistribution as percent injected dose per gram tissue (% ID/g) at 2, 4, and 6 hours post injection of ^{125}I-IgG and ^{111}In-Tropolone leukocytes in normal mice

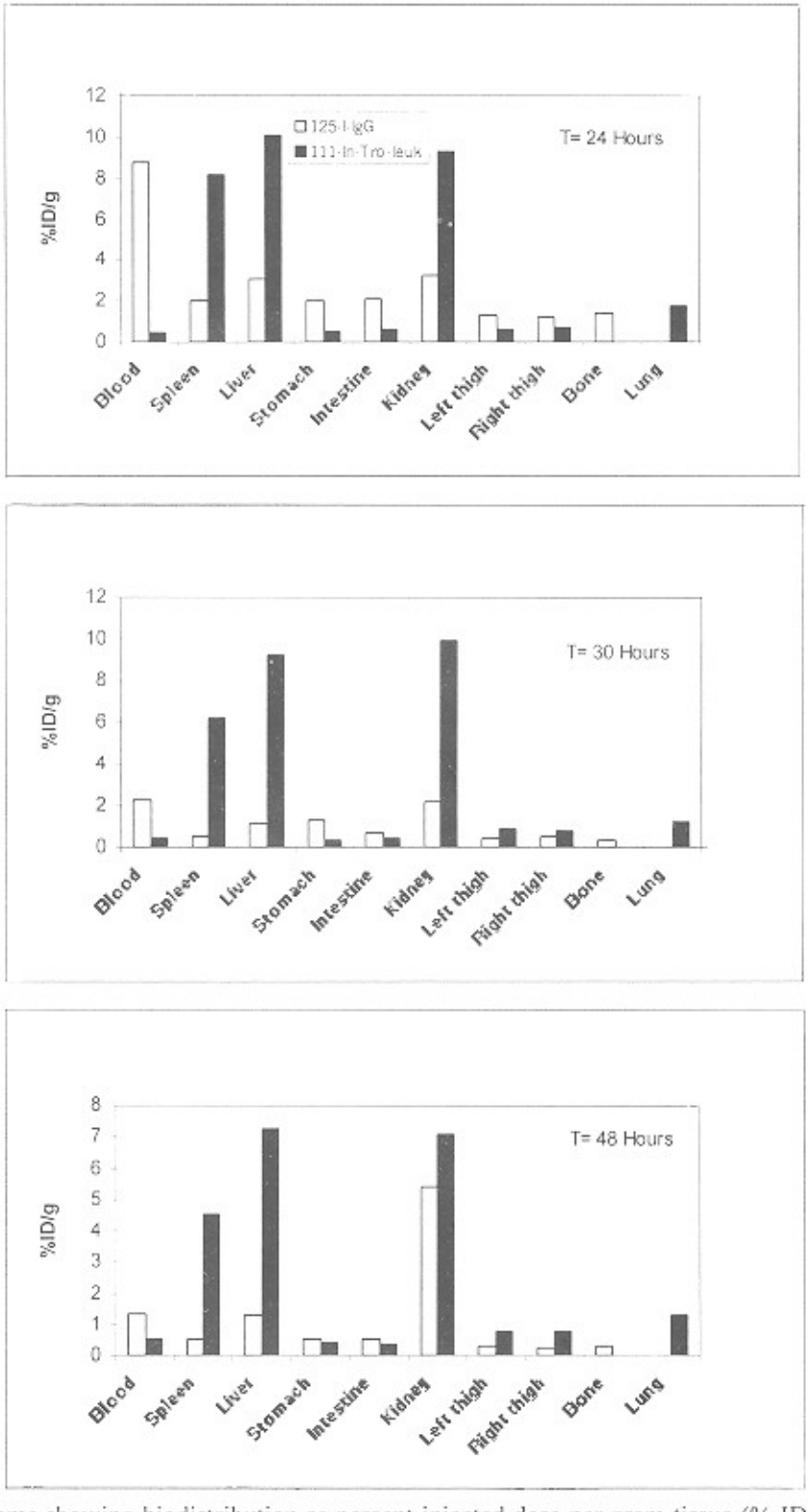

Fig 2. Histograms showing biodistribution as percent injected dose per gram tissue (% ID/g) at 24, 30, and 48 hours post injection of ^{125}I IgG and ^{111}In-Tropolone leukocytes in normal mice

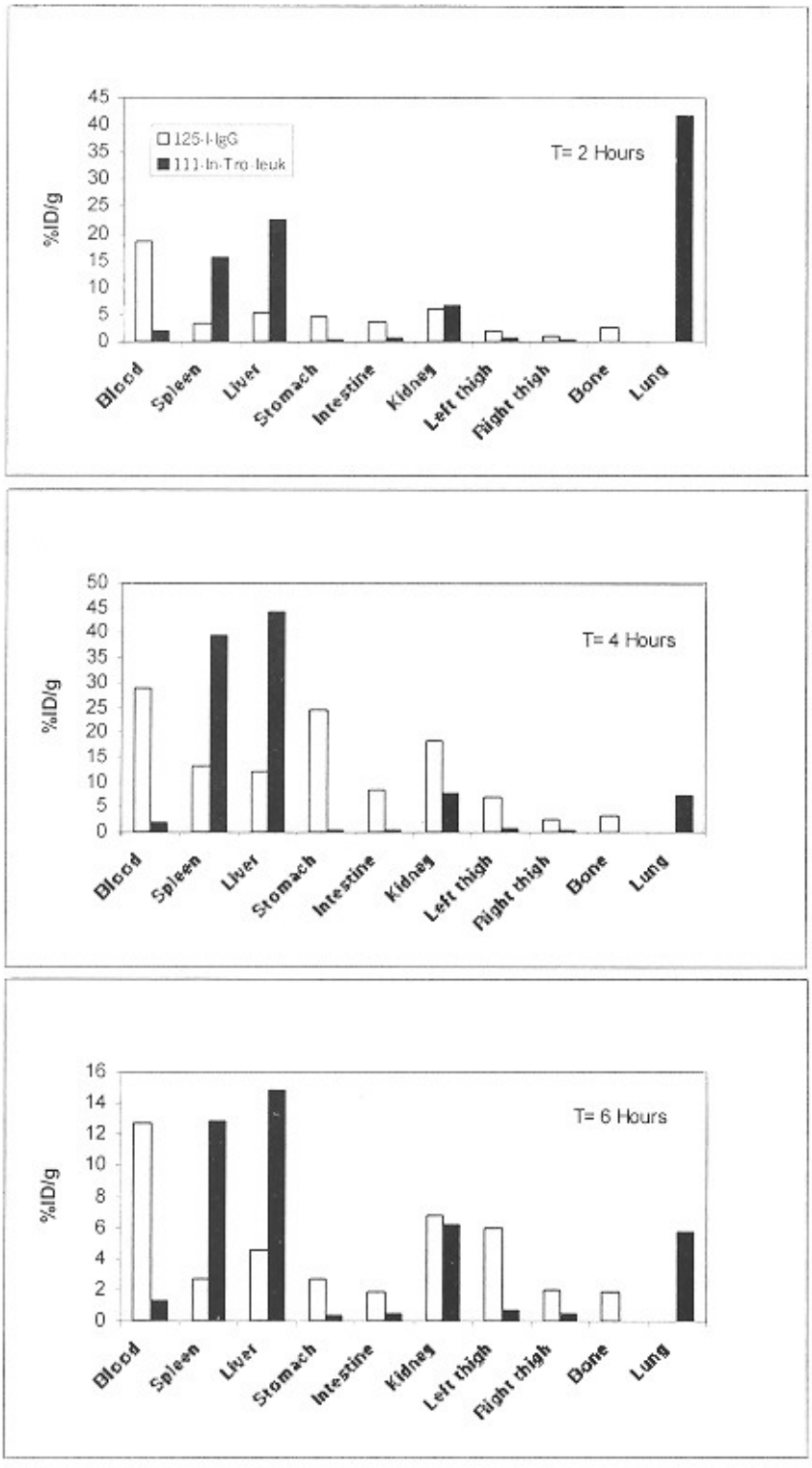

Fig 3. Histograms showing biodistribution as percent injected dose per gram tissue (% ID/g) at 2, 4, and 6 hours post injection of ^{125}I-IgG and ^{111}In-Tropolone leukocytes in induced inflammation mice

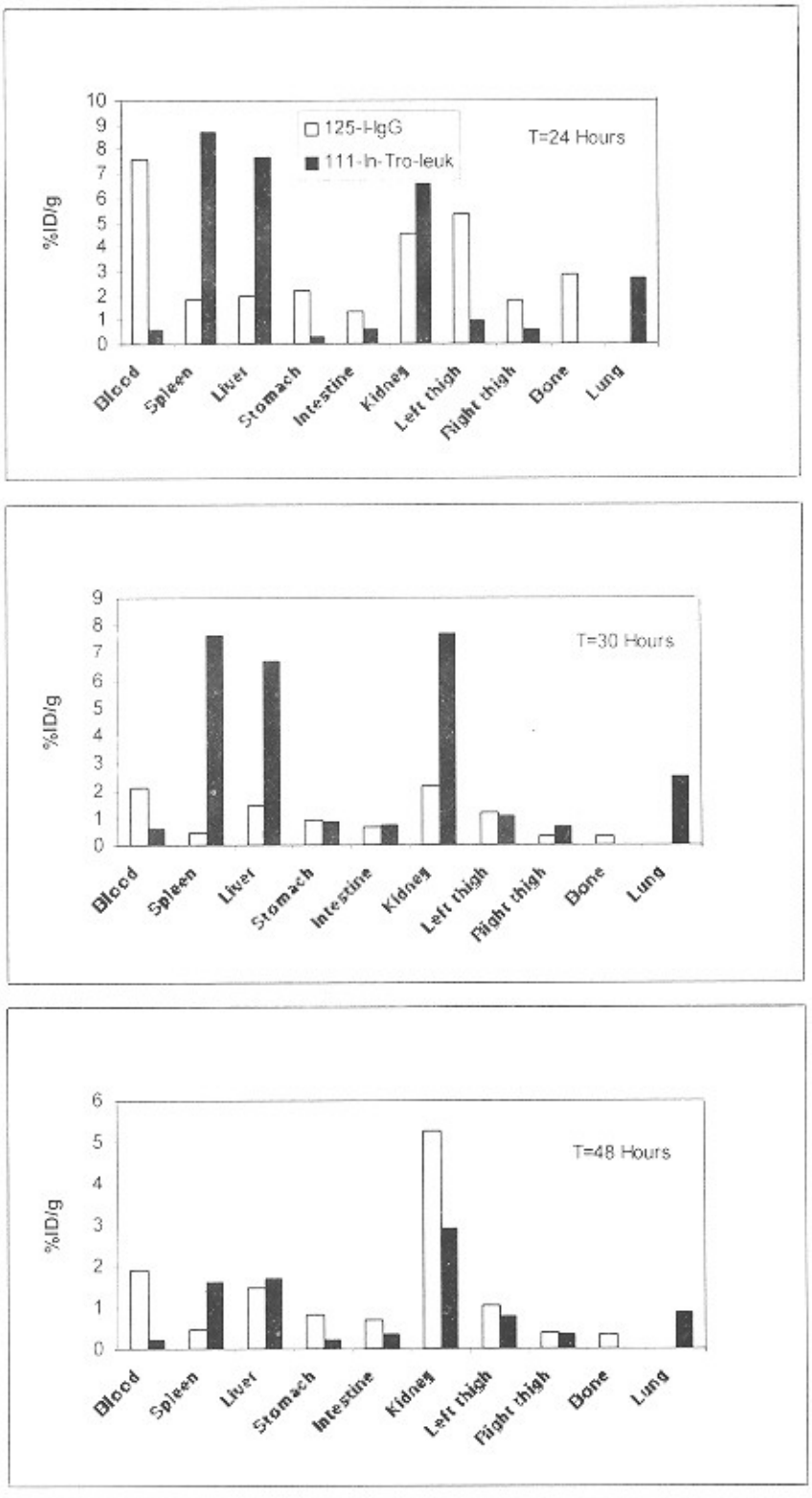

Fig 4. Histograms showing biodistribution as percent injected dose per gram tissue (% ID/g) at 24, 30, and 48 hours post injection of ^{125}I-IgG and ^{111}In-Tropolone leukocytes in induced inflammation mice

Table 1: % ID/g in normal mice at 2, 4, 6, 24, 30, 48 hr post injection of ^{125}I-IgG

Organ	2 hr	4 hr	6 hr	24 hr	30 hr	48 hr
Blood	14.40±4.48	26.7±4.3	17±2.94	8.8±0.1	2.3±0.5	1.32±0.72
Spleen	4.2±0.36	5.23±0.76	3.4±0.71	2.03±0.5	0.48±0.13	0.52±0.16
Liver	6.6±1.15	7.3±0.8	5.85±0.68	3.1±0.85	1.16±0.57	1.25±0.4
Stomach	6.13±0.98	13.77±4.81	5.48±1.95	2.03±0.29	1.34±0.39	0.51±0.2
Intestine	3.15±1.19	5.95±0.35	2.85±0.5	2.08±0.37	0.66±0.22	0.53±0.12
Kidney	7.05±0.57	23.85±3.85	6.8±1.06	3.2±0.29	2.2±0.57	5.42±0.11
Left Thigh	1.43±0.45	2±0.5	1.53±0.28	1.35±0.05	0.43±0.18	0.28±0.1
Right Thigh	1.5±0.31	2.3±0.36	1.25±0.18	1.25±0.05	0.56±0.19	0.22±0.05
Bone	2.1±0.54	2.67±0.19	1.57±0.21	1.4±0.25	0.31±0.07	0.28±0.03

(%ID) percent injected dose per gram tissue.

Table 2: % ID/g in induced inflammation mice at 2, 4, 6, 24, 30, 48 hr post injection of ^{125}I-IgG

Organ	2 hr	4 hr	6 hr	24 hr	30 hr	48 hr
Blood	18.57±3.04	28.82±3.53	12.75±1.64	7.58±0.8	2.13±0.32	1.93±0.64
Spleen	3.33±1.08	13.17±1.72	2.63±0.62	1.83±0.33	0.49±0.26	0.49±0.21
Liver	5.15±1.31	12.1±2.72	4.6±1.37	1.97±0.7	1.44±0.59	1.47±0.12
Stomach	4.68±1.11	24.63±5.75	2.63±0.7	2.2±0.57	0.92±0.45	0.82±0.4
Intestine	3.5±0.54	8.42±1.81	1.9±0.3	1.32±0.33	0.68±0.32	0.68±0.24
Kidney	5.8±0.73	18.1±3.82	6.8±1.74	4.53±0.78	2.18±0.84	5.27±1.86
Left Thigh	2±0.54	6.87±0.69	6±1.22	5.35±0.65	1.2±0.28	1.03±0.69
Right Thigh	1±0.48	2.69±1.12	2±0.52	1.77±0.06	0.35±0.06	0.39±0.15
Bone	2.81±0.29	3.28±0.4	1.83±0.35	2.83±0.66	0.34±0.07	0.36±0.1

(%ID) percent injected dose per gram tissue.

DISCUSSION

The use of radiolabeled nonspecific polyclonal IgG to image sites of inflammation has been shown to be due to nonspecific accumulation of the protein resulting from increased vascular permeability. The clinical results for immunoscintigraphy with radiolabeled granulocyte-specific antibodies and human nonspecific polyclonal IgG indicate their potential value for the detection of infectious and inflammatory processes (1,7). However, a general difficulty in the use of radiolabeled antibodies is the excessive accumulation in normal organs such as liver and the slow clearance of the label from circulation. To overcome this, several methods have been proposed such as splitting up antibodies into bivalent or monovalent fragments or reducing to just the hypervariable region (8), administration of a second antibody (9), pretargeting concept (10-12), and altering the radiolabeling methods. Most of investigations have been focused on improvement radiolabeling methods (13). Different radionuclides (^{111}In, ^{99m}Tc, ^{123}I) have been employed to radiolabel antibodies. Short physical half-life of ^{99m}Tc, dehalogenation of radioiodine, and sequestration of ^{111}In in reticuloendothelial organs particularly the liver, are disadvantages of these radionuclides (14,15). Although ^{123}I with half-life 13 hr and single gamma with 159 Kev (83-85 %) is the radionuclide of choice for most imaging tracer studies, transportation problems and expense have limited the use of ^{123}I radionuclide.

In this study, we labeled human nonspecific polyclonal IgG with Na^{125}I, which is a cheap, widely available, and easily detectable radionuclide. Following administration of radioiodinated antibodies, in vivo deiodination happens. Deiodination increases radioactivity in blood, stomach, intestine, kidney, and decreases the target-to-nontarget radioactivity ratios (13). As shown in Figures 3-4, labeled IgG accumulated in the inflammation site better than labeled leukocytes. There was significant difference at definite times between the radioactivity of left and right thigh after injection of both ^{125}I-IgG and ^{111}In-Tropolone leukocytes in induced inflammation mice. Following administration of labeled leukocytes, blood levels were 7-14 times lower ($P<0.05$) relative to those of labeled IgG, but activity levels in liver, spleen,

and kidney were higher with respect to the labeled IgG. Because of the reduced blood levels with labeled leukocytes at all time points, improved inflammatory thigh-to-blood ratios were apparent at all times, whereas inflammatory thigh-to-liver, spleen, and kidney ratios were low.

Table 3: % ID/g in normal mice at 2, 4, 6, 24, 30, 48 hr post injection of ^{111}In-tropolone leukocytes

Organ	2 hr	4 hr	6 hr	24 hr	30 hr	48 hr
Blood	1.94±0.185	1.465±0.498	1.834±0.286	0.430±0.181	0.404±0.132	0.512±0.332
Spleen	13.286±0.71	40.098±0.129	24.55±0.136	8.152±0.821	6.214±0.132	4.512±0.332
Liver	37.421±0.945	55.894±0.520	38.721±0.815	10.1±0.721	9.21±0.152	7.23±0.521
Stomach	0.939±0.618	0.452±0.175	0.352±0.100	0.506±0.306	0.374±0.127	0.404±0.175
Intestine	0.370±0.045	0.486±0.104	0.826±0.179	0.594±0.206	0.454±0.088	0.327±0.098
Kidney	10.627±0.583	10.360±0.196	13.554±0.201	9.266±0.087	9.877±0.957	7.088±0.504
Left thigh	0.627±0.174	0.642±0.265	0.845±0.025	0.639±0.171	0.843±0.030	0.729±0.093
Right Thigh	0.424±0.228	0.660±0.229	0.886±0.024	0.715±0.127	0.812±0.054	0.773±0.185
Lung	37.832±0.308	7.004±0.453	2.600±0.12	1.715±0.721	1.216±0.112	1.266±0.185

(%ID) percent injected dose per gram tissue.

Table 4: % ID/g in Induced Inflammation Mice at 2,4,6, 4,30,48 hr post injection of ^{111}In- tropolone leukocytes

Organ	2 hr	4 hr	6 hr	24 hr	30 hr	48 hr
Blood	2.014±0.562	1.693±0.504	1.284±0.077	0.584±0.142	0.606±0.087	0.229±0.051
Spleen	15.593±3.905	39.279±0.959	12.847±0.410	8.692±2.286	7.642±0.515	1.628±1.047
Liver	22.452±2.413	44.087±1.035	14.784±0.926	7.700±2.328	6.677±5.601	1.697±0.845
Stomach	0.433±0.116	0.297±0.041	0.374±0.156	0.287±0.041	0.857±0.717	0.220±0.105
Intestine	0.520±0.126	0.542±0.122	0.477±0.129	0.593±0.143	0.705±0.143	0.335±0.076
Kidney	6.516±1.802	7.520±1.820	6.234±0.617	6.565±2.780	7.692±2.426	2.93±1.421
Left thigh	0.645±0.072	0.678±0.098	0.675±0.017	0.925±0.186	1.064±0.012	0.782±0.182
Right thigh	0.473±0.075	0.534±0.084	0.419±0.026	0.550±0.063	0.645±0.078	0.331±0.093
Lung	41.529±0.850	7.333±0.808	5.691±0.746	2.689±0.677	2.483±1.525	0.863±0.081

(%ID) percent injected dose per gram tissue.

Table 5: Inflammatory (left) thigh-to-normal tissue ratios in inflammation bearing mice after injection of ^{125}I-IgG and ^{111}In-Tropolone-leukocytes. a: unmeasured values

Organ	2 hr		4 hr		6 hr		24 hr		30 hr		48 hr	
	^{125}I-IgG	^{111}In-leu	^{125}I-IgG	^{111}In-leu	^{125}I-IgG	^{111}In-leu	^{125}I-IgG	^{111}In-leu	^{125}I-IgG	^{111}In-leu	^{125}I-IgG	^{111}In-leu
Blood	0.1	0.32	0.23	0.4	0.47	0.52	0.7	1.58	0.56	1.75	0.53	3.4
Spleen	0.6	0.04	0.52	0.017	2.28	0.052	2.9	0.1	2.4	0.13	2.1	0.48
Liver	0.3	0.02	0.56	0.015	1.3	0.045	2.7	0.12	0.83	0.15	0.7	0.46
Stomach	0.4	1.48	0.27	2.28	2.28	1.8	2.43	3.22	1.3	1.24	1.25	3.5
Intestine	0.57	1.24	0.81	1.25	3.15	1.4	4.05	1.55	1.76	1.5	1.5	2.3
Kidney	0.34	0.098	0.37	0.09	0.88	0.1	1.18	0.14	0.55	0.13	0.19	0.26
Right Thigh	2	1.36	2.55	1.26	3	1.61	3.02	1.68	3.42	1.64	2.66	2.36
Bone	0.7	a -	2.09	-	3.27	-	1.89	-	3.5	-	0.9	-
Lung	-	0.015	-	0.092	-	0.11	-	0.34	-	0.42	-	0.9

Following administration of ^{125}I-IgG, levels of activity in most normal tissues, except blood, were lower compared with the ^{111}In-Tropolone-leukocytes. On the other hand the ratio of inflammatory thigh-to-normal tissues except blood were better for labeled IgG than labeled leukocytes. Based on results, inflammation can be visualized within 24 and 48 hr after injection of ^{125}I-IgG and ^{111}In-Tropolone-leukocytes, respectively; furthermore, inflammation is more separable than background after injection of ^{125}I-IgG compared with ^{111}In-Tropolone-leukocytes. Due to diffusion of lipophilic complex of Tropolone with ^{111}In into any cell, it is necessary to isolate leukocytes from whole blood before labeling. The production is time consuming and

needs skilled personnel and expensive laboratory facilities. In addition, since the procedure is carried out on the patient's own blood (4) there are also risks of hepatitis and acquired immune deficiency syndrome (AIDS). Therefore, because of better localization of labeled IgG in inflammatory sites, low uptake in reticuloendothelial system, better target-to-background ratio, and simple labeling method compared to labele leukocytes, it is suggested antibody scintigraphy be more suitable agent than labeled leukocytes for detection of inflammation in a mouse model.

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REFERENCES

1. Corstens, F.H.M., Oyen, W.J.G., Becker, W.S. (1993) Radiommunoconjugates in the detection of infection and inflammation. Semin. Nucl. Med. 23: 148-164.
2. Corstens, F.H.M., Claessens, R.A.M.J. (1992) Imaging inflammation with human polyclonal immunoglobulin: Not looked for but discovered. Eur. J. Nucl. Med. 19: 155-158.
3. Chatal, J.F., Peltier, P., Bardies, M., Chetanneau, A., Thedrez, P., Faivre-Chauvet, A. (1992) Does immunoscintigraphy serve clinical needs effectively? Is there a future for radioimmunotherapy? Eur. J. Nucl. Med. 19(3): 205-213.
4. Danpure, H. J., Osman, S. (1994) Radiolabeling of blood cells- Theory and Methodology. In: Sampson, C.B. (ed). Textbook of Radiopharmacy (Theory and Practice). In: Cox, P.H., Clinic, D.D.H. (eds). Nuclear Medicine: A series of Monographs and Texts. 2nd edn, Gordon & Breach Science Publishers, Amsterdam, pp: 69-86.
5. Johnston, A., Thorpe, R. (1996) Purification of immunoglobulins, constituent chains and fragments. In: Immunochemistry in practice. 3rd edn, Blackwell Science, Oxford, pp: 62-65.
6. Johnston, A., Thorpe, R. (1996) Radiolabeling techniques. In: Immunochemistry in Practice. 3rd edn, Blackwell Science, Oxford, pp: 134-136.
7. Lind, P., Langsteger, W., Koltringer, P., Dimai, H.P., Passl, R., Eber, O. (1990) Immunoscintigraphy of inflammatory processes with a technetium-99m-labeled monoclonal antigranulocyte antibody (Mab BE 250/183). J. Nucl. Med. 31: 417-423.
8. Fazio, F., Giovanni, P. (1993) Antibody-guided scintigraphy: targeting of the "magic bullet". Eur. J. Nucl. Med. 20: 1138-1140.
9. Goodwin, D., Mears, C., Diamanti, C., McCall, M., Lai, C., Torti, F., McTigue, M. (1984) Use of specific antibody for rapid clearance of circulating blood background from radiolabeled tumor-imaging proteins. Eur. J. Nucl. Med. 9(5): 209-215.
10. Goodwin, D.A., Mears, C.F., McCall, M.J., McTigue, M., Chaovapong, W. (1988) Pre-targeted immunoscintigraphy of murine tumors with indium-111-labeled bifunctional haptens. J. Nucl. Med. 29: 226-234.
11. Kalofonos, H.P., Rusckiwski, M., Siebecker, D.A., Sivolapenko, G.B., Snook, D., Lavender, J.P., Epenetos, A.A., Hnatowich, D.J. (1990) Imaging of tumor in-patients with indium-111-labeled biotin and streptavidin-conjugated antibodies: preliminary communication. J. Nucl. Med. 31: 1791-1796.
12. Goldenberg, D.M. (1993) Monoclonal antibodies in cancer detection and therapy. Am. J. Med. 94: 297-312.
13. Zimmer, A.M. (1996) New approaches to radiolabeling monoclonal antibodies. In: Henkin, R.E., Boles, M.A., Dillehay, G.L., Halama, J.R., Karesh, S.M., Wagner, R.H., 14. Zimmer, A.M., (eds). Nuclear Medicin. Masby-Year Book, St Louis, pp: 511-515.
14. Rayudu, G.V.S. (1990) Production of radionuclides for medicine. Semin. Nucl. Med. 20(2): 100-110.
15. Britton, K.E., Granowska, M., Mather, S.J. (1991) Radiolabeled monoclonal antibodies in oncology I. Technical aspects. Nucl. Med. Commun. 12: 65-76.