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Original Article

Tissue Distribution of ^{125}I-human Nonspecific Polyclonal IgG in Normal and Induced Inflammation Mice

Soraya Shahhosseini*[a], Mohammad Hossein Babaei[b], Reza Najafi[b]

[a]*Department of Pharmacetical Chemistry, School of Pharmacy, Shaheed Beheshti University of Medical Sciences and Health Services, Tehran, Iran.* [b]*Radioisotopes Division, Nuclear Research Center, Atomic Energy Organization of Iran, Tehran, Iran.*

Abstract

Many different radiolabeled antibodies have been used for radioimmunotherapy and radioimmunoscintigraphy of human diseases in animal experiments. In order to study the in vivo tissue distribution of antibody, we labeled human nonspecific polyclonal IgG with Na^{125}I using chloramine-T method. An animal model was developed by injecting turpentine in the posterior left thigh of Balb/c mice. Tissue distribution of ^{125}I-IgG was assessed in normal and induced inflammation mice. Although labeled IgG accumulated in normal tissues such as liver, spleen and blood, its localization in inflammatory left thigh was significantly different from normal right thigh. Thus, by using the radiolabeling methods of IgG in order to increase the target-to-background ratio, antibody scintigraphy could be suggested as a powerful diagnostic tool for inflammation.

Keywords: Immunoscintigraphy; Turpentine; Immunoglobulin (Ig); Percent Injected Dose per gram tissue (% ID/g).

Introduction

Various antibodies have been labeled with different radionuclides and used for radioimmunoimaging (1-6). The most important limitation of this application is that the labeled antibodies accumulate not only in the target but also in normal tissues (nontarget). To overcome this, several methods have been proposed (7-11). Most of the investigations are focused on improving radiolabeling methods (12) resulting in reduction of background activity and enhancement of target-to-background ratio. Radiolabeled leukocytes and a few radiopharmaceuticals such as ^{67}Ga-citrate and ^{99m}Tc-labeled antigranulocytes antibody preparations can be used to detect infectious and inflammatory foci (13). Since human nonspecific polyclonal IgG accumulates in inflammatory and infectious foci, in this paper, we studied the tissue distribution of whole IgG labeled with Na^{125}I using chloramine-T method in normal and induced inflammation mice.

* Corresponding author:
E-mail: sshahhosseini@irimc.org

Experimental

^{125}I as Na^{125}I with specific activity of 100 µci/µl in NaOH was purchased from Amersham. IgG was prepared from human plasma (14) and was iodinated by Chloramin-T method (15). 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. Specific activity of ^{125}I- IgG was estimated to be between 5-10 mci/mg.

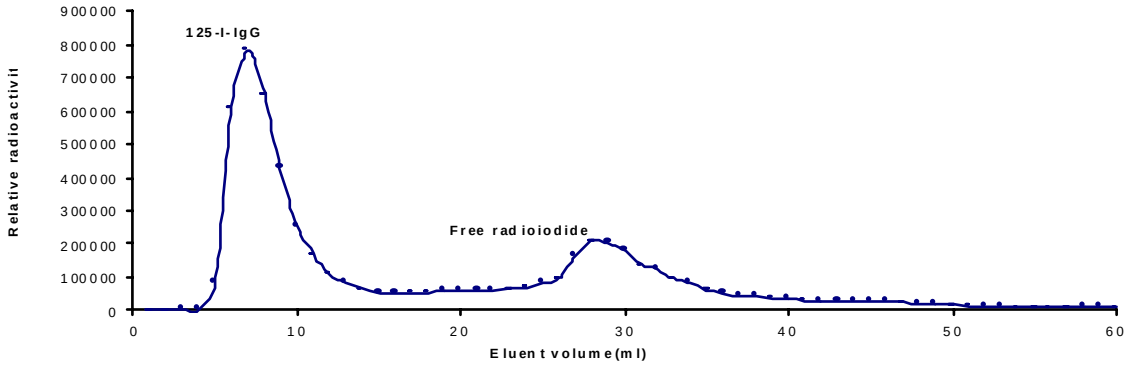

Figure 1. Gel filtration of labeled protein (Sephadex G-25)

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 h in normal condition to develop the inflammation foci. 100 µci / 0.1 ml / 20 µg ^{125}I -IgG / 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 h post administration of ^{125}I-IgG. Selected organs (blood, spleen, liver, stomach, intestine, kidney, left thigh, right thigh, and bone) 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 $P < 0.05$.

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

Organ	2 h	4 h	6 h	24 h	30 h	48 h
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

Data are expressed as percent injected dose per gram. Mean ±s.d. for 6 mice

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

Organ	2 h	4 h	6 h	24 h	30 h	48 h
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

Data are expressed as percent injected dose per gram. Mean ±s.d. for 6 mice

Results and Discussion

The result of separation of free radioactive iodine from labeled IgG by Gel filtration chromatography is shown in figure 1. Labeling efficiency and specific activity were 75% and 5-10 µci / µg respectively.

The results of tissue distribution in normal and induced inflammation mice at 2, 4, 6, 24, 30 and 48 h post injection of ^{125}I-IgG (n=6 per time point) are shown in figures 2 and 3.

There was no significant difference between the radioactivity of left and right thigh in mice without inflammation after injection of ^{125}I -IgG (Table1). In inflammation bearing mice, radioactivity values of left and right thigh were significantly different ($P<0.05$) at definite times (6, 24, 30, and 48 h) after injection of ^{125}I -IgG (Table 2).

Table 3. Inflammatory (left) thigh-to-normal tissue ratios in inflammation bearing mice after injection of ^{125}I –IgG

Organ	2 h	4 h	6 h	24 h	30 h	48 h
Blood	0.1	0.23	0.47	0.7	0.56	0.53
Spleen	0.6	0.52	2.28	2.9	2.4	2.1
Liver	0.3	0.56	1.3	2.7	0.83	0.7
Stomach	0.4	0.27	2.28	2.43	1.3	1.25
Intestine	0.57	0.81	3.15	4.05	1.76	1.5
Kidney	0.34	0.37	0.88	1.18	0.55	0.19
Right thigh	2	2.55	3	3.02	3.42	2.66
Bone	0.7	2.09	3.27	1.89	3.5	0.9

The maximum radioactivity of normal tissues and inflammatory (left) thigh was achieved 4 h after injection of ^{125}I -IgG, and reduced dramatically after 4 h (e.g. the % ID/g blood felled from ~29% at 4 h to 13% at 6 h and 7.5% at 24 h). The reduction of radioactivity in inflammatory (left) thigh was not as much as the reduction of radioactivity in

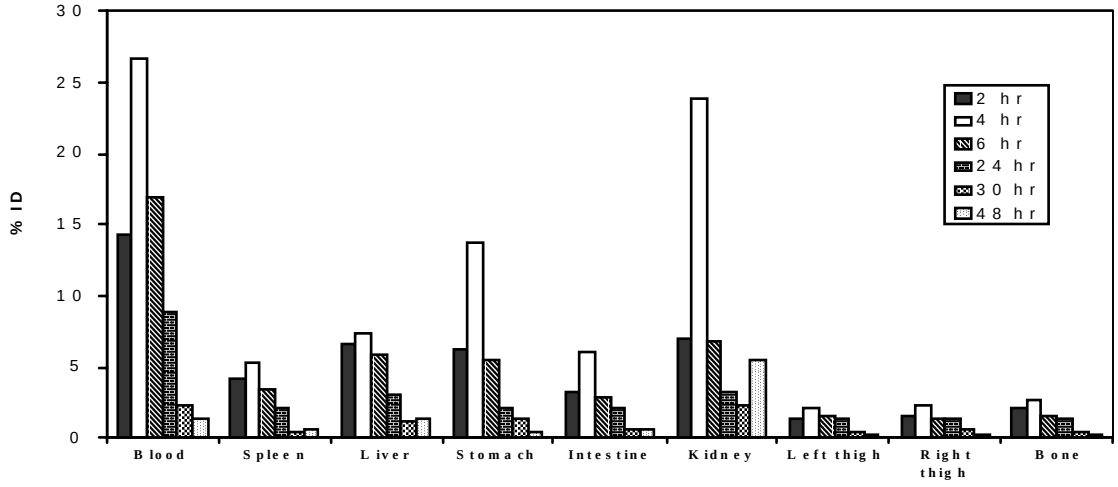

Figure 2. Histograms showing biodistribution (% ID/g) at 2, 4, 6, 24, 30, and 48 hours post injection of ^{125}I -IgG in normal mice

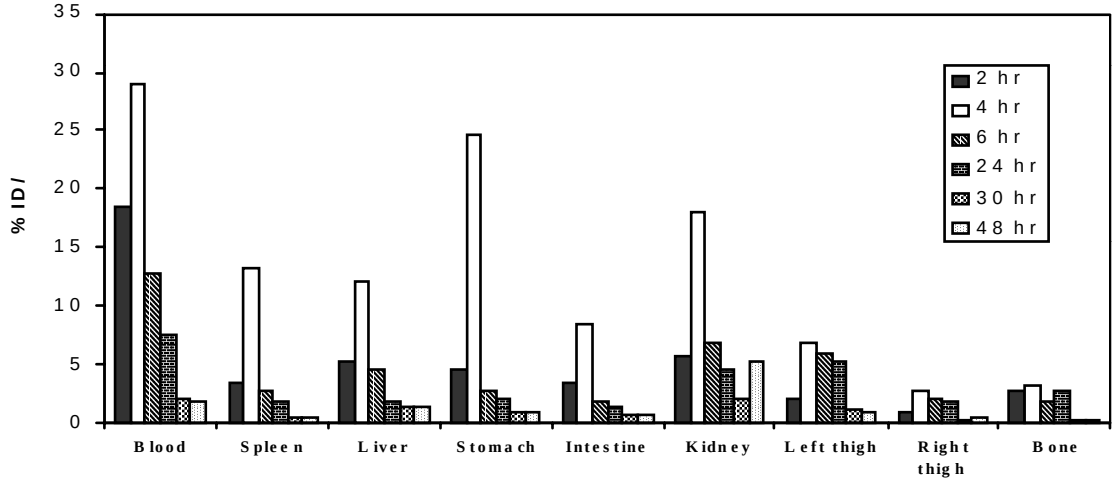

Figure 3. Histograms showing biodistribution (% ID/g) at 2, 4, 6, 24, 30, and 48 hours post injection of ^{125}I -IgG in induced inflammation mice

normal tissues (the % ID/g dropped from 7% at 4 h to 6% at 6 h and 5% at 24 h). The best inflammatory thigh-to-normal tissue ratios were obtained at 24hr post administration of ^{125}I -IgG (Table 3).

Many studies using antibodies have utilized radioiodine (^{125}I, ^{123}I, ^{131}I) as the tracer to follow the distribution of antibodies in animals (16-21). The beta emission and poor imaging characteristics of ^{131}I have limited its use. Although ^{123}I with half-life of 13 h and single gamma with 159 Kev (83-85 %) is the radionuclide of choice for most imaging tracer studies, transportation problems and cost have limited the use of ^{123}I radionuclide. ^{125}I, a cheap, widely available, and easily detectable radionuclide, has been used mainly as a label for biodistribution studies (22). In this study, we labeled human nonspecific polyclonal IgG with Na^{125}I by chloramine-T method, which is an efficient method for the direct substitution of ^{125}I into the tyrosyl residues of protein (23).

As shown in Figure 3, labeled IgG is accumulated in the inflammation site. There was significant difference at definite times between the radioactivity of left and right thigh after injection of ^{125}I-IgG in induced inflammation mice. Levels of activity in most normal tissues, were high and based on the results, inflammation can be visualized within 24 h after injection of ^{125}I-IgG (Table1). Following administration of radioiodinated antibodies, in vivo deiodination occurs. Deiodination increases radioactivity in blood, stomach, intestine, kidney, and decreases the target-to-nontarget radioactivity ratios (12). In spite of localization of ^{125}I-IgG in normal tissues, its accumulation in inflammatory site is high enough to be recognized from background. Investigations have shown that the accumulation of IgG in inflammatory and infectious foci results from increased vascular permeability (24). The clinical results for immunoscintigraphy with radiolabeled human nonspecific polyclonal IgG indicate the potential value of this method for the detection of infectious and inflammatory processes (25-26). However, a general difficulty in the application 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 the antibodies into bivalent or monovalent fragments or reducing to just the hypervariable region (7), administration of a second antibody (8), pretargeting concept (9-11), and altering the radiolabeling methods.

Therefore, due to the high localization of labeled IgG in inflammatory sites, if radiolabeling methods of antibodies are improved, background radioactivity decreases, then inflammation would be more recognizable from background in images. Although, much remains to be done to insure the clinical usefulness of this approach, it is suggested that radiolabeled antibody is more a suitable method compared to other methods as radiopharmaceutical for the localization of inflammation.

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