LOCALIZATION OF ^{99m}Tc-LABELLED NONSPECIFIC HUMAN IMMUNOGLOBULIN AND GALLIUM-67 CITRATE IN INFLAMMATORY PROCESS IN THE MICE

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Imaging inflammation with ^{67}Ga or radiolabelled autologous leucocytes is an established nuclear medicine modality. Recently polyclonal immunoglobulin labelled with ^{99m}Tc has shown clinical utility for inflammation detection, but the localization process is little understood. ^{99m}Tc-hIg and ^{67}Ga localize in sites of inflammation by different mechanisms. Acute infections, for example, appear to be detected well with ^{111}In-hIg and ^{111}In-WBCs, while chronic infection seems to be readily detected with ^{67}Ga.

The aim of this study was to compare the ability of polyclonal hIg coupled to MDP labelled with ^{99m}Tc and ^{67}Ga to concentrate at focal sites of inflammation induced by chemical stimuli to mice.

The reduced hIg was prepared using controlled reduction reaction to generate -SH groups, presumibly the high affinity, low capacity sites, through incubation of antibody with a reducing agent, β-mercaptoethanol. ^{99m}Tc antibody labelling was carried out under homogeneous phase conditions, using tin MDP kit to reduce pertechnetate (Matter, SJ et al, *J.Nucl.Med. 30:*692-7,1990). The radiolabelling efficiency and ^{99m}Tc antibody stability were controlled by paper chromatographic systems.

MATERIAL AND METHODS

- Preparation of instant one vial reduced hIg kit. Lyophilized hIg (Sandoglobulin) was dissolved in sterile, 0.9% saline. Ten mg of hIg with sufficient β-mercaptoethanol (molar ratio 1000:1) was incubated at room temperature for 30 min. The reduced antibody was purified by gel filtration on a 20 ml Sephadex G-50 fine column and eluted using phosphate buffered saline as the mobile phase. The vials containing 500 µg of reduced antibody, 40 µg of MDP, 2.75 µg of SnF_2 and 8 µg of PABA were prepared as a lyophilized kit. Approximately 1 mCi of ^{99m}Tc (from IPEN-TEC generator) in 1 ml of normal saline was added to each vial to be radiolabelled.
- Gallium-67 citrate. ^{67}Ga (IPEN/SP, carrier free) was diluted with 3.8 % (w/v) sodium citrate to 1 mCi/ml.
- Biodistribution study. Each Swiss mouse, female with 2 months of age was administered IM in the right thigh with 40 µl of turpentine to produce focal inflammation. The 100 µl of the solution containing the ^{99m}Tc-hIG (10-30 µg, approximately 100 µCi) or ^{67}Ga (100 µCi) were administered through the tail

vein (IV) into a group of mice bearing inflammatory lesions induced previously by turpentine, 24 hr for acute process and 150 hr for chronic process.At time intervals of 4 and 24 hrs postinjection, groups of mice were killed and samples of blood (100 µl), the entire liver, spleens, heart, stomach with contents, kidneys, left and right legs, bowel (only for ^{67}Ga groups) were dissected and counted in a well counter against a standard of the injectate. The results were expressed as mean percentage of injected dose per organ.Each group was compared with normal mice group.

RESULTS AND DISCUSSION

^{99m}Tc-hIG

	4 hrs			24 hrs		
ORGAN	Normal	Acute	Chronic	Normal	Acute	Chronic
Heart	0.54±0.04	0.59±0.12	0.60±0.13	0.24±0.05	0.21±0.03	0.20±0.04
Lungs	3.36±0.87	3.30±1.72	2.38±0.73	1.32±0.42	0.94±0.24	1.27±0.32
Kidneys	7.03±0.48	7.35±0.65	6.78±0.86	3.57±0.38	4.35±0.67	4.26±0.46
Liver	6.74±0.33	10.84±0.49*	9.07±1.13	3.82±0.47	5.81±0.66*	6.09±0.74*
Spleen	0.34±0.18	0.51±0.07	0.46±0.12	0.45±0.60	0.23±0.07	0.17±0.01
Stomach	1.27±0.36	1.76±1.50	0.62±0.11	0.46±0.08	1.13±0.72*	1.80±0.80*
Right leg		2.78±0.33	2.78±1.18		1.82±0.34	1.49±0.24
Left leg(1)		11.07±4.86	6.12±1.82		10.22±4.32	3.66±0.83
Blood(2)	32.86±5.51	58.78±4.86*	29,06±3.23*	14.30±2.84	20.75±2.30*	18.28±2.10
Abs/Liver		1.02±0.21	0.62±0.09		1.70±0.78	0.58±0.18
Abs/blood		0.19±0.06	0.22±0.08		0.48±0.15	0.20±0.05
Abs/right leg		4.08±1.25	2.26±0.65		5.76±1.99	2.26±0.65
		^{67}Ga-citrate				
Heart	0.21±0.07	0.27±0.03	0.29±0.04*	0.16±0.04	0.11±0.02	0.09±0.01*
Lungs	1.01±0.18	1.31±0.33	1.10±0.40	0.66±0.16	0.56±0.07	0.33±0.03*
Kidneys	1.80±0.50	2.40±0.28	3.29±0.52*	5.43±0.65	3.11±0.59	1.69±0.93*
Liver	15.13±1.44	6.92±1.43*	29.70±2.92*	18.65±2.46	8.75±1.31*	7.33±1.43*
Spleen	0.45±0.17	1.32±0.35*	1.34±0.28*	0.90±0.29	0.55±0.32*	2.14±0.27*
Stomach	1.09±0.31	0.49±0.09*	0.82±0.26	2.03±0.70	1.07±0.36*	1.00±0.10*
Bowel	1.64±0.33	2.10±0.31*	1.14±0.37	5.28±0.97	1.11±0.73*	5.95±0.72
Right leg	3.54±0.55	3.16±0.79	2.76±0.55*	4.70±0.76	2.16±1.50	1.27±0.40*
Left leg(1)		11.93±2.19	8.67±2.05		4.95±1.54	1.97±0.02
Blood(2)	20.05±0.49	16.32±1.80*	17.12±1.99*	7.67±1.94	4.67±1.53*	3.88±0.91*
Abs/liver		0.39±0.07	0.29±0.07		0.63±0.20	0.29±0.06
Abs/blood		0.70±0.19	0.47±0.07		1.14±0.59	0.57±0.14
Abs/right leg		3.83±0.32	2.97±1.07		2.29±0.58	1.87±0.53

Left leg (1) = abscess; Blood (2) = 7% of body weight; * = Significance of difference of Student's test, P>0.05.

Table above shows the tissue distributions (% injected dose/organ ± sd) at 4 and 24 hr post-injection of ^{67}Ga and ^{99m}Tc-hIG into mice bearing turpentine induced. Experimental evidence indicates that the mouse model we have developed is reliable and the turpentine induced inflammatory lesions should be satisfactory for the uptake study ot ^{99m}Tc-hIG and ^{67}Ga. Both the acute and chronic processes, with ^{99m}Tc-hIG, the abscess-to-right leg ratios were similar between 4 and 24 hrs while with ^{67}Ga, the abscess-to-right leg ratios were similar to ^{99m}Tc-IG at 4 hr and relatively smaller at 24 hr than ^{99m}Tc-IG.