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Comparison of Tc-99m HIG and Ga-67 citrate in the evaluation of bacterial abscess in a rat model

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Tc-99m labeled polyclonal human immunoglobulin (HIG) has been shown to be able to localize an inflammatory site. There are several possible explanations for HIG accumulation at focal infection sites such as increased vascular permeability, binding of the Fc part of Ig to Fc receptors of leucocytes and binding directly to bacteria. In this study, we compared Tc-99m HIG and Ga-67 citrate scintigraphy in localizing acute bacterial abscesses induced by *E. coli* and *S. aureus*. Serial scintigrams were performed at 1, 4, 24 hr after injection. Tc-99m HIG showed greater accumulation at all times with both infectious agents than Ga-67 citrate ($p < 0.05$). While Tc-99m HIG showed greater accumulation in *S. aureus* than *E. coli* ($p < 0.05$), there was no statistically significant difference between *E. coli* and *S. aureus* ($p > 0.05$) by Ga-67 citrate. Our study suggests that Tc-99m HIG is a superior agent to Ga-67 and bacterial affinity can be a factor responsible for HIG accumulation at focal sites of inflammation.

Key words: Tc-99m HIG, Ga-67 citrate, abscess

INTRODUCTION

INFECTION is an important cause of morbidity and mortality. In many instances, demonstration of the anatomic site of the process is more important than identifying the microbial content of the abscess.[1] Early in the evaluation of an inflammatory lesion, before formation of an abscess, conventional radiography, computed tomography (CT) and ultrasonic scanning (US) can not readily localize the area of involvement while radionuclide imaging with gallium-67 (Ga-67) citrate or labeled leucocytes has been relatively successful for the detection of early inflammation.[2] But these techniques have required 24 hr between injection and imaging to detect sites of inflammation. Recently, it has been demonstrated that polyclonal nonspecific human immunoglobulin (HIG) labeled with either Tc-99m or In-111 is an effective agent for focal sites of inflammation.[3–5] The mechanisms responsible for this accumulation at focal infection sites have not yet been clarified, but several possible explanations such as increased vascular permeability, binding of the Fc part of Ig to Fc receptors on leucocytes and, binding to microorganisms at the focal infection site have been implicated.[2,6–8]

In this study, we aimed to evaluate and compare Tc-99m HIG and Ga-67 citrate scintigraphy in localizing acute bacterial infection, induced by *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).

MATERIALS AND METHODS

Animal model

The appropriate microbial strain of *E. coli* and *S. aureus* was incubated overnight on tripticase soy agar plates at 37°C. Individual colonies were diluted with sterile normal saline to produce a turbid suspension containing ~3×10^9 organisms/m*l*.

Thirty-two Wistar Albino rats, weighing ~150 g were anesthetized with ether and their left thigh muscles were traumatized by pinching with a hemostat. Then 0.1 m*l* of a suspension containing ~3×10^9 organisms/m*l*. *E. coli* was injected into the traumatized thigh muscle of 16 rats. Sixteen other rats were infected in the same way with a 0.1 m*l* of a suspension containing ~3×10^9 /m*l* *S. aureus*.

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Table 1 The distrubition of rats according to infection and imaging agent

Group	n	Infection agent	Imaging agent
1	8	*E. coli*	Tc-99m HIG
2	8	*S. aureus*	Tc-99m HIG
3	8	*E. coli*	Ga-67 citrate
4	8	*S. aureus*	Ga-67 citrate

Twenty four hours later, when gross swelling was apparent in the infected thigh, the radiolabeled reagents were injected intravenously via the tail vein.

Radiopharmaceuticals and imaging

Human nonspecific polyclonal immunoglobulin (Mallinckrodt Diagnostica, Holland) was radiolabeled with Tc-99m as previously reported[5] and 0.1 mg HIG/37 MBq Tc-99m/0.2 m*l* for each rat was injected into 16 rats (8 of them were infected with *E. coli* and the other 8 rats with *S. aureus*). 7.4 MBq/0.2 m*l* Ga-67 citrate for each rat was injected into another 16 rats (8 of them were infected with *E. coli* and the other 8 rats with *S. aureus*). The rats were divided into four groups according to the infection and imaging agents (Table 1).

The animals were anesthetized with ether and imaged at 1, 4 and 24 hr after injection of the radiolabeled agents. Images were recorded for a preset time of 10 min.

All scintigrams were performed with a Toshiba GCA 602 A digital gamma camera. At the conclusion of imaging, the anesthetized rats were killed, inflammation was confirmed by histopathological examination (Fig. 1) and the causative microorganisms were recultured.

Data analysis

The images were analyzed by placing a region of interest over the total lesion area (total lesion activity-TLA), over a comparable zone of the contralateral normal thigh (contralateral thigh activity-CLTA) and over the entire body of the rat (Total Body Activity-TBA) (Fig. 2). Two parameters were calculated as follows:

(1) The relative uptake (RU):

$$\frac{TLA - [(CLTA/pixel) \times NPL]}{(CLTA/pixel) \times NPL}$$

(2) The percent residual activity (RA%):

$$\frac{TLA - [(CLTA/pixel) \times NPL]}{TBA} \times 100$$

NPL: Number of pixels in the lesion

Mann Whitney U and Wilcoxon tests were used for statistical analysis. Results are shown as the mean ± SD.

Table 2 RU values in all groups

Group	n	1 hr	4 hr	24 hr
HIG *E. coli*	8	1.16 ± 0.16*	2.52 ± 0.25*	4.05 ± 0.54*
HIG *S.aureus*	8	1.72 ± 0.40**	3.00 ± 0.53**	4.46 ± 0.85**
Ga-67 *E. coli*	8	0.91 ± 0.13†	2.08 ± 0.08†	3.00 ± 0.14†
Ga-67 *S. aureus*	8	0.94 ± 0.20††	2.05 ± 0.16††	2.96 ± 0.26††

*p < 0.05, **p < 0.05, †p < 0.05, ††p < 0.05

Table 3 RA% values in all groups

Group	n	1 hr	4 hr	24 hr
HIG *E. coli*	8	1.08 ± 0.14*	2.27 ± 0.17*	2.65 ± 0.14*
HIG *S. aureus*	8	1.58 ± 0.26**	2.60 ± 0.41**	3.35 ± 0.64**
Ga-67 *E. coli*	8	0.87 ± 0.15†	2.05 ± 0.13†	2.46 ± 0.15†
Ga-67 *S. aureus*	8	0.90 ± 0.15††	1.95 ± 0.12††	2.42 ± 0.10††

*p < 0.05, **p < 0.05, †p < 0.05, ††p < 0.05

RESULTS

RU and RA% values are shown in Table 2 and Table 3, respectively. The RU and RA% values at 1, 4 and 24 hr increased continuously in all groups. Tc-99m HIG images were superior to Ga-67 images at all imaging times (Figs. 3 and 4). The maximum RU and RA% values were detected at 24 hr by both radionuclides but RU and RA% values for Tc-99m HIG were significantly higher at all times than for Ga-67 citrate ($p < 0.05$). Moreover, RU and RA% values were considerably increased in the *S. aureus* compared to the *E. coli* group in Tc-99m HIG scintigraphy ($p < 0.05$), whereas there was no significant difference between these groups in Ga-67 citrate scintigraphy ($p > 0.05$) (Figs. 5 and 6).

DISCUSSION

Radiolabeled human polyclonal IgG readily accumulates in inflammatory lesions to an extent sufficient to yield excellent external images.[2,9] The nonspecificity of this agent is suggested by two types of observations. First, a wide variety of inflammatory processes, ranging from those due to both gram-negative and gram-positive bacteria, and secondly those due to a yeast or a nonmicrobial chemical irritant were imaged equally well.[2] There are several possible explanations for HIG accumulation at a focal infection site: (1) Increased vascular permeability,[2] (2) Binding Fc part of Ig to Fc receptors of leucocytes,[8] (3) Binding directly to bacteria.[10]

Breitz et al.[11] showed that there was no statistically significant difference between In-111 HIG and Tc-99m HSA uptake in sterile abscesses induced by turpentine. On the other hand, another study showed that In-111 HIG uptake was higher than Tc-99m HSA uptake in abscesses induced by *E. coli*.[6] Our study also showed that Tc-99m HIG accumulation increased continuously at 1, 4 and 24

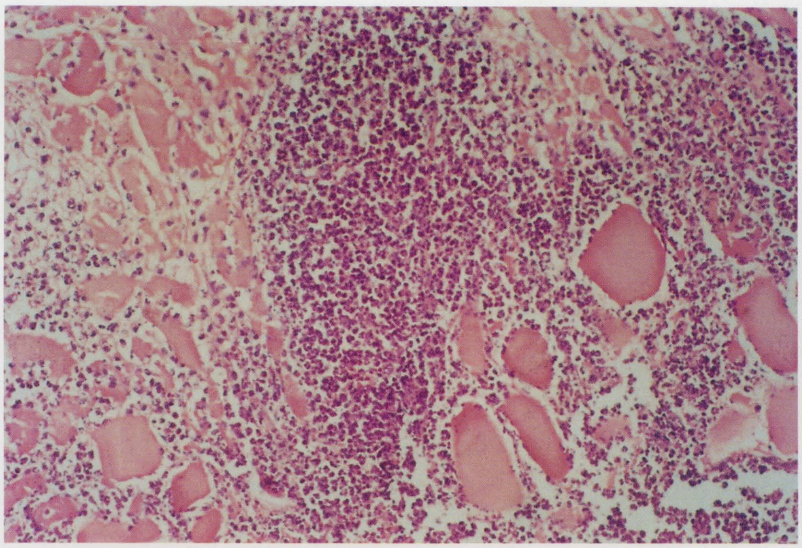

Fig. 1 Photomicrograph showed neutrophil dominant inflammatory cells infiltration in the striated muscle tissue (20 ×; hematoxylin eosin).

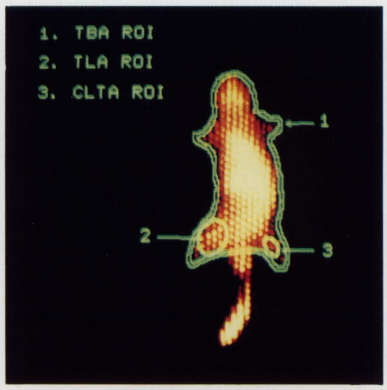

Fig. 2 Three ROIs were shown. 1. Total Body Activity ROI: TBA ROI, 2. Total Lesion Activity ROI: TLA ROI, 3. Contralateral Thigh Activity ROI: CLTA ROI.

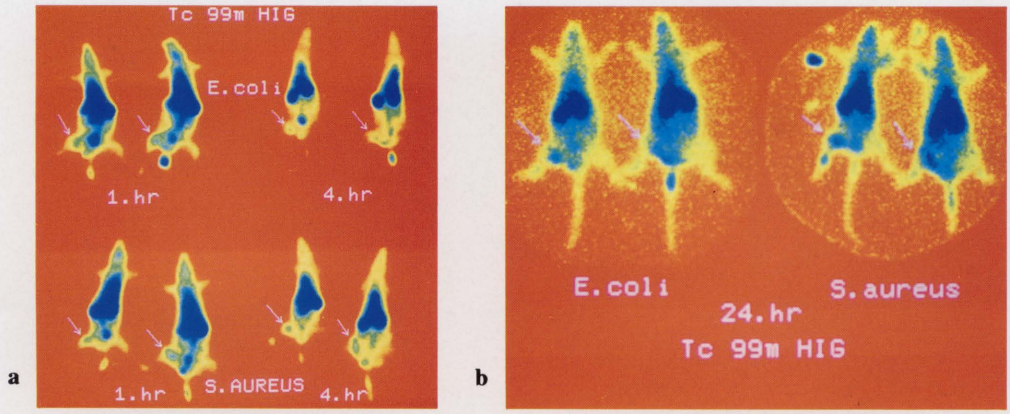

Fig. 3 Tc-99m HIG images. a. Arrows show abscess site in the left thigh induced *E. coli* (upper row) and *S. aureus* (lower row). b. Prominent accumulation of Tc-99m HIG at 24 hr with both infection agent.

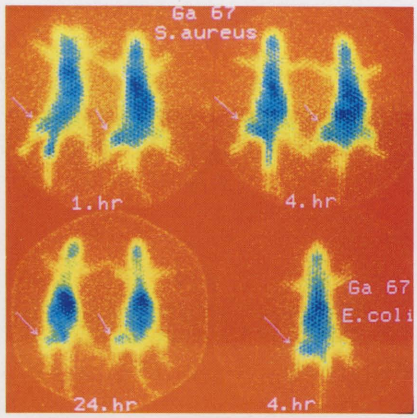

Fig. 4 Ga-67 citrate images: 1, 4, 24 hr *S. aureus* images and 4 hr *E. coli* image (lower right corner).

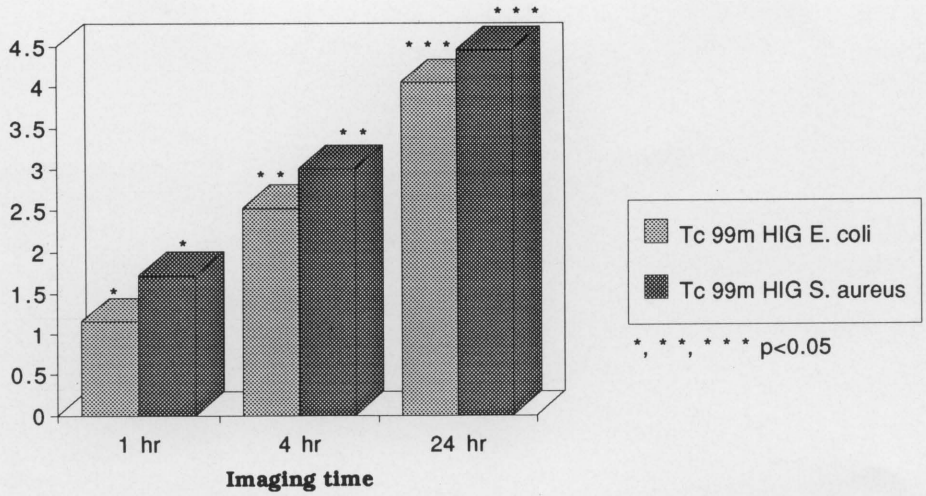

Fig. 5 Greater RU values with *S. aureus* than *E. coli* by Tc-99m HIG.

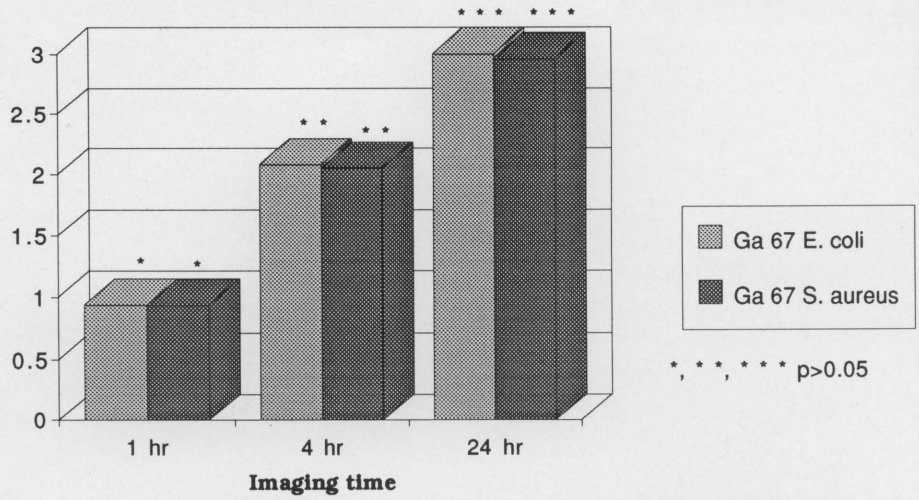

Fig. 6 Similar RU values with both infectious agents by Ga-67 citrate.

hr. Although Saptogino et al.[12] indicate that HIG is bound predominantly to monocytes (74%), to granulocytes (12%) and to red blood cells (14%); Calame et al.[13] showed that there was no statistically significant difference between leucocytopenic and normal animals for Tc-99m HIG accumulation at an infection site. These results suggest that bacterial affinity can be as important a factor as vascular permeability and binding of the Fc part of IgG to Fc receptors on leucocytes.

An *in vitro* study showed that Tc-99m HIG uptake was superior in *S. aureus* to that in *E. coli*.[10] Greater *in vitro* accumulation of HIG in *S. aureus* was thought to be due to the protein A content of *S. aureus* cell membrane which bound to the Fc part of IgG, since Tc-99m HIG showed more affinity in Cowan 1 type *S. aureus* which has more protein A content than EMS type *S. aureus*.[10]

In our *in vivo* study, RU and RA% values were higher in the *S. aureus* than *E. coli* group in Tc-99m HIG scintigraphy ($p < 0.05$), whereas there was no statistically significant difference, between these groups in Ga-67 citrate scintigraphy.

On the other hand, we showed that RU and RA% values were higher with Tc-99m HIG in both microbial agents than with Ga-67 citrate. This result confirmed that Tc-99m HIG was a superior agent to Ga-67. Rubin et al.[6] also showed that greater accumulation of In-111 HIG was present in abscesses. Greater RU and RA% values in Tc-99m HIG scintigraphy when compared with Ga-67 are due to the differences between the biodistribution characteristics of the two radionuclides. Blood clearance of Ga-67 was slower than Tc-99m HIG. Saptogino et al.[14] showed that approximately 7% of the injected activity was observed in the blood pool but 25% for Ga-67.[15] Bone and muscle activities can be responsible factors affecting RU and RA% values in abscesses in thigh localization. Approximately 1.8% of the administered dose was retained in the bone at 5 hr and 2.2% at 24 hr for Ga-67, against 0.6% and 0.2%, respectively, for Tc-99m HIG. The biodistribution of both radionuclides in muscle was similar.

Whereas approximately 0.3% of the administered dose was retained in the muscle at 5 hr and 0.1% at 24 hr for Ga-67; the values were 0.2% and 0.1%, respectively, for Tc-99m HIG. Whereas the effective dose equivalent for Ga-67 citrate was 0.11 mSV/MBq; it was 0.008 mSV/MBq for Tc-99m HIG.[14] On the other hand, Ga-67 scintigraphy obscured abdominal lesions because of bowel excretion of Ga-67 citrate; whereas no bowel excretion occurs for 24 hr in Tc-99m HIG scintigraphy.[14] For these reasons, Tc-99m HIG is a more valuable agent than Ga-67 citrate.

In conclusion, our study suggests that Tc-99m HIG is a superior agent to Ga-67 citrate for localizing inflammation foci, and bacterial affinity can be as responsible a factor for HIG accumulation as other factors.

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