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Biodistribution of ^{111}In-labelled IgG and IgM in experimental infection

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Summary

Both the protein used and the conjugation method are factors which may be relevant for targeting infection with ^{111}In-labelled proteins. In this study, human immunoglobulin G (IgG), conjugated to either DTPA or LiLo, and LiLo conjugated human immunoglobulin M (IgM) were evaluated. In rats with *Staphylococcus aureus* calf muscle infection, biodistribution was determined 6, 24 and 48 h after the injection of ^{111}In-DTPA-IgG, ^{111}In-LiLo-IgG or ^{111}In-LiLo-IgM. Absolute abscess uptake of ^{111}In-LiLo-IgG was significantly higher than that of ^{111}In-DTPA-IgG ($P < 0.05$). Since blood clearance of ^{111}In-LiLo-IgG was initially significantly slower ($P < 0.01$), the higher abscess uptake did not result in higher abscess-to-background ratios. ^{111}In-LiLo-IgG accumulated to a greater extent in the liver ($P < 0.001$). ^{111}In-DTPA-IgG showed higher uptake in the kidneys and bone marrow ($P < 0.001$ and $P < 0.01$, respectively). Although decreasing over time, ^{111}In-LiLo-IgM showed reasonable abscess uptake and rapid blood clearance, resulting in higher abscess-to-background ratios compared with ^{111}In-LiLo-IgG ($P < 0.01$). However, liver and spleen uptake were three- to four-fold higher than that of ^{111}In-LiLo-IgG ($P < 0.001$). Compared with DTPA-conjugation, chelation with LiLo has a minor influence on abscess targeting of ^{111}In-labelled IgG. However, differences in blood clearance and organ uptake do occur. ^{111}In-LiLo-IgM shows high relative accumulation in abscesses as well as high liver and spleen uptake. ^{111}In-LiLo-IgM appears promising for imaging infection outside the trunk region.

Introduction

A number of studies have demonstrated the utility of ^{111}In-labelled non-specific polyclonal human immunoglobulin G (^{111}In-IgG) scintigraphy for the detection of various types of focal infection in humans [1, 2]. In a clinically used formulation, the protein is conjugated with the bifunctional chelating agent diethylenetriamine-pentaacetic (DTPA) cyclic anhydride for labelling with ^{111}In. However, the chelator may play an important role in the distribution of the radiolabel in various tissues [3]. Release of ^{111}In from the conjugates produces a continuous leakage of radionuclide into the blood, resulting in transport through transferrin to the liver and other organs of the mononuclear phagocytic system [4]. It has been suggested that 1,3-bis[*N*-[*N*-(2-aminoethyl)-2-aminoethyl]-2-aminoacetamido]-2-4-(isothiocyanatobenzyl) propane-*N*, *N*, *N'*, *N''*, *N'''*, *N''''*, *N'''''*, *N''''''*-octaacetic acid (LiLo) forms a kinetically more stable complex for binding ^{111}In to proteins [3]. In the first part of the study, IgG conjugated to either DTPA or LiLo and subsequently labelled with ^{111}In was studied in a rat model of infection.

Not only may the ligand be relevant for biodistribution, but the protein may also play an important role [5]. As reported previously, proteins smaller than IgG (such as human serum albumin) accumulate in a similar fashion to IgG in experimental infection. However, the background activity is higher, due to the higher influx into the non-target tissues [5, 6], resulting in lower abscess-to-background ratios of albumin compared with IgG [5]. In the second part of the study, IgM (molecular weight 900 kDa, which is five times larger than IgG) was radiolabelled with ^{111}In after LiLo conjugation and compared with ^{111}In-LiLo-IgG.

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Materials and methods

Radiopharmaceuticals

^{111}In-DTPA-IgG. The bifunctional chelating agent DTPA bicyclic anhydride was conjugated to IgG (Sandoglobulin, Sandoz AG, Nürnberg, Germany) as described previously [7]. Conjugation of DTPA to IgG resulted in two to three DTPA ligands per protein molecule.

^{111}In-LiLo-IgG and ^{111}In-LiLo-IgM. As described earlier, the bifunctional chelating agent LiLo was conjugated to IgG (Sandoglobulin, Sandoz AG, Nürnberg, Germany) and to the human monoclonal IgM antibody 16.88, directed against an intracellular epitope in adenocarcinoma cells (PerImmune Inc./Akzo Nobel, Rockville, MD, USA) [3]. Conjugation of LiLo to the proteins resulted in three to four LiLo ligands per protein molecule.

Radiolabelling and dosing

Aliquots of 0.5 mg of the conjugate were radiolabelled with ^{111}In (Indium chloride, Mallinckrodt Medical, Pette, The Netherlands) via citrate transchelation [5]. The radiochemical purity of the radiolabelled proteins was determined by instant thin-layer chromatography (ITLC) on Gelman ITLC-SG strips (Gelman Laboratories, Ann Arbor, MI) with 0.1 M citrate (pH = 5) as solvent. The labelling efficiency was always higher than 95%. A dose of 10 μg, labelled with 2 MBq ^{111}In, was injected intravenously.

Animals and study design

In young, male, randomly bred Wistar rats (weight 200–220 g), a calf muscle abscess was induced after ether anaesthesia with approximately 2×10^8 colony-forming units of *Staphylococcus aureus* in 0.1 ml of a 50:50 (v/v) suspension of autologous blood and normal saline. The animals were randomly divided into groups. Twenty-four hours after the inoculation of *S. aureus* in the muscle, when swelling of the muscle was apparent, the respective radiopharmaceuticals were injected via the tail vein.

For tissue collection, the rats were killed with 30 mg phenobarbital injected intraperitoneally, followed by cervical dislocation at 6, 24 and 48 h post-injection. Each protein was evaluated in five animals at each time point. Samples of bone marrow (taken from the right femur) and blood were collected. The infected left calf muscle, the right calf muscle, liver, spleen, kidneys, lung, duodenum and the right femur were dissected and blotted dry. The activity in the tissues and samples was measured in a shielded well-type gammacounter. To correct for radioactive decay and permit calculation of the uptake of the radiopharmaceuticals in each organ as a fraction of the injected dose, aliquots of the respective doses were counted simultaneously. The activity measured in the tissues and samples was expressed as a percentage of the injected dose per gram (% i.d. g^{-1}) and the excreted activity per animal as a percentage of the total dose administered.

For calculation of background activity, we used a combination of normal muscle activity and blood activity as described previously [5]. Since the blood volume of rats approximates to 60 ml per kg body weight (6%), 94% × the activity per gram in muscle plus 6% × the activity per gram in blood was adopted as the value for background activity [8]. Abscess-to-background ratios were also calculated.

Statistical analysis

All mean values are given as % i.d. g^{-1} or ratios ± one standard error of the mean (S.E.M.). Statistical analysis was performed using one-way analysis of variance (ANOVA). The level of significance was set at $P < 0.05$.

Results

Comparison of ^{111}In-DTPA-IgG and ^{111}In-LiLo-IgG

As shown in Table 1, conjugation of IgG with LiLo resulted in a marginally higher amount of abscess uptake of ^{111}In-LiLo-IgG compared with ^{111}In-DTPA-IgG. Only at 48 h post-injection was a significant difference observed ($P < 0.05$). As shown in Fig. 1, abscess-to-background ratios were similar at all time points. At 6 and 24 h post-injection, blood levels of ^{111}In-LiLo-IgG were significantly higher ($P < 0.001$–$P < 0.01$). As indicated in Table 2, ^{111}In-LiLo-IgG showed relatively high liver uptake ($P < 0.001$), while the renal uptake of ^{111}In-DTPA-IgG was significantly higher ($P < 0.001$–$P < 0.01$). In spleen, lungs, bowel and initial bone marrow, the uptake of both agents was similar. At 48 h post-injection, bone marrow uptake of ^{111}In-DTPA-IgG was slightly, but significantly, higher ($P < 0.05$).

Comparison of ^{111}In-LiLo-IgG and ^{111}In-LiLo-IgM

As shown in Table 1, the uptake of ^{111}In-LiLo-IgM in the abscess was significantly lower compared with that of ^{111}In-LiLo-IgG ($P < 0.001$–$P < 0.01$). While the ^{111}In-LiLo-IgG concentration in the abscess remained constant, significant washout of ^{111}In-LiLo-IgM was observed over time ($P < 0.05$). ^{111}In-LiLo-IgM cleared significantly faster from the blood than ^{111}In-DTPA-IgG ($P < 0.001$). Due to this faster background clearance, the abscess-to-background ratios of ^{111}In-LiLo-IgM increased with time and were significantly higher than the ratios obtained with ^{111}In-LiLo-IgG ($P < 0.001$). As indicated in Table 2, ^{111}In-LiLo-IgM was in the main taken up in liver and spleen ($P < 0.001$), while renal, pulmonary and initial bowel uptake were significantly lower than those of

Table 1. Mean (± S.E.M.) abscess, muscle and blood activity (% i.d. g^{-1}) and abscess-to-background ratio 6, 24 and 48 h post-injection (p.i.).

	Time p.i. (h)	*^{111}In-DTPA-IgG*	*^{111}In-LiLo-IgG*	*^{111}In-LiLo-IgM*
Abscess	6	1.08 ± 0.05	1.21 ± 0.08 ^{b}	0.78 ± 0.06
	24	1.09 ± 0.07	1.22 ± 0.11 ^{b}	0.64 ± 0.07
	48	1.09 ± 0.03 ^{a}	1.34 ± 0.10 ^{c}	0.57 ± 0.05
Muscle	6	0.13 ± 0.01	0.11 ± 0.01	0.06 ± 0.01
	24	0.16 ± 0.02	0.14 ± 0.02	0.03 ± 0.01
	48	0.16 ± 0.01	0.17 ± 0.02	0.03 ± 0.01
Blood	6	2.58 ± 0.09 ^{c}	3.20 ± 0.09 ^{c}	1.37 ± 0.23
	24	1.11 ± 0.04 ^{b}	1.53 ± 0.10 ^{c}	0.13 ± 0.03
	48	0.69 ± 0.04	0.99 ± 0.14 ^{c}	0.02 ± 0.01
Abscess-to-background ratio	6	3.94 ± 0.24	4.16 ± 0.29	6.63 ± 1.62
	24	4.42 ± 0.46	5.14 ± 0.52 ^{b}	14.46 ± 2.48
	48	5.99 ± 0.29	6.20 ± 0.29 ^{b}	19.69 ± 3.69

$^aP < 0.05$; $^bP < 0.01$; $^cP < 0.001$.

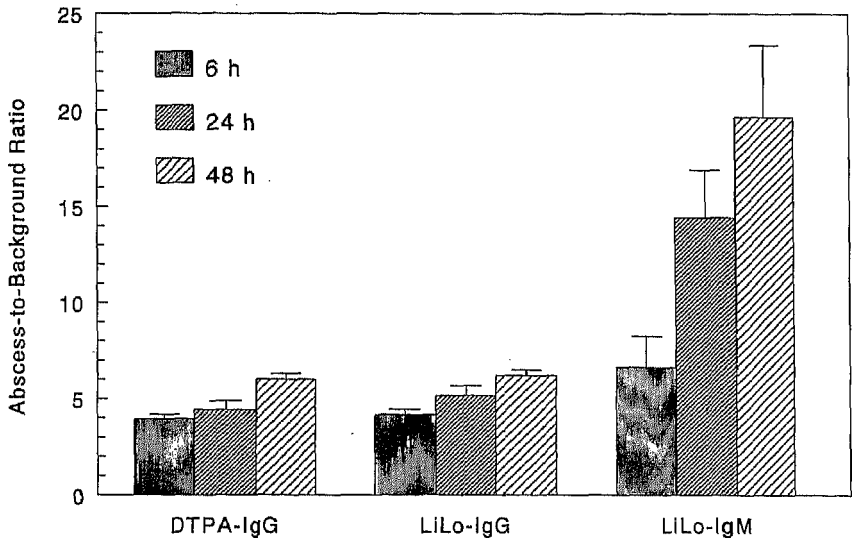

Fig. 1. Abscess-to-background ratios (the error bars indicate the S.E.M.

^{111}In-LiLo-IgG ($P < 0.001$–$P < 0.05$). The bone marrow uptake of both radiopharmaceuticals was similar.

Discussion

The first aim of this study was to evaluate the possible advantage of using a more stable chelating agent than DTPA, such as LiLo [3], for ^{111}In labelling of IgG. DTPA- and LiLo-conjugated IgG showed similar abscess uptake and abscess-to-background ratios, indicating a more stable ^{111}In-labelled complex is not necessarily better suited for the detection of abscesses. It would appear that the stability of ^{111}In-DTPA-IgG is sufficiently high to enable adequate abscess accumulation. It has been shown that the dissociation and subsequent entrapment of radionuclide in the extracellular matrix is an important factor in ^{111}In uptake in experimental abscesses after the injection of ^{111}In-DTPA-IgG, while the protein or protein fragments are split off and washed out [9, 10]. Therefore, more stable ^{111}In chelation will not automatically result in higher abscess uptake. The increased stability of the LiLo complex may be reflected in the slower blood clearance and lower bone marrow uptake. Uptake in organs was also affected: ^{111}In-LiLo-IgM accumulated to a greater extent in liver, whereas ^{111}In-DTPA-IgG showed

Table 2. Mean (± S.E.M.) tissue uptake (% i.d. g^{-1}) 6, 24 and 48 h post-injection (p.i.).

	Time p.i. (h)	*^{111}In-DTPA-IgG*		*^{111}In-LiLo-IgG*		*^{111}In-LiLo-IgM*
Liver	6	0.90 ± 0.02	c	1.90 ± 0.07	c	4.33 ± 0.23
	24	0.91 ± 0.05	c	2.11 ± 0.02	c	5.43 ± 0.24
	48	1.02 ± 0.02	c	2.77 ± 0.40	c	4.97 ± 0.17
Spleen	6	1.16 ± 0.06		1.26 ± 0.03	c	4.40 ± 0.59
	24	1.40 ± 0.06		1.41 ± 0.08	c	3.66 ± 0.24
	48	1.56 ± 0.06		1.80 ± 0.14	c	3.33 ± 0.10
Kidney	6	3.67 ± 0.13	c	2.29 ± 0.21	c	1.16 ± 0.08
	24	3.80 ± 0.14	b	2.99 ± 0.23	c	1.12 ± 0.05
	48	3.75 ± 0.07	c	2.66 ± 0.16	c	1.07 ± 0.05
Lung	6	0.98 ± 0.03		1.09 ± 0.06	c	0.49 ± 0.04
	24	0.54 ± 0.01		0.64 ± 0.04	c	0.11 ± 0.02
	48	0.43 ± 0.01		0.51 ± 0.05	c	0.08 ± 0.01
Bone marrow	6	1.23 ± 0.09		1.10 ± 0.08		1.16 ± 0.09
	24	0.99 ± 0.11		0.74 ± 0.14		0.78 ± 0.06
	48	1.17 ± 0.03	a	0.83 ± 0.06		0.78 ± 0.10
Bowel	6	0.36 ± 0.02		0.36 ± 0.02	a	0.28 ± 0.02
	24	0.27 ± 0.01		0.26 ± 0.01	a	0.22 ± 0.01
	48	0.23 ± 0.01		0.23 ± 0.01		0.20 ± 0.02

$^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

higher renal uptake. Our data in rats with infection concur with previous studies in tumour-bearing athymic mice in which four different ^{111}In-chelate–antibody complexes showed similar tumour uptake but a very different distribution in organs [4]. Other studies have indicated that the most striking differences between radiometal–DTPA–antibody complexes and more stable chelates occur after the first 48 h post-injection [11, 12].

The second aim of the study was to evaluate the possible advantage of using a protein larger than IgG for the detection of infection. Striking differences were observed when we compared ^{111}In-LiLo-IgG with ^{111}In-LiLo-IgM. Although absolute abscess uptake of IgM was lower, abscess-to-background ratios were up to three times higher due to the very fast clearance from the background. As IgM is relatively large compared with IgG (900 *vs* 150 kDa), one might assume that penetration into an abscess would be more difficult. Although the absolute uptake of IgM was as much as half that of IgG, it should be sufficiently high to make adequate imaging of foci possible. In contrast to the uptake of ^{111}In in an abscess after ^{111}In-LiLo-IgG injection, which remains relatively stable over time, ^{111}In-LiLo-IgM uptake decreased in the abscess. This can be explained by the relatively fast clearance from blood and background compared with IgG, allowing ^{111}In-LiLo-IgM washout from the abscess. These findings are similar to those in previous studies with ^{111}In-DTPA-IgA [5]. ^{111}In-DTPA-IgA cleared even faster from the blood pool to liver, spleen and also kidneys than ^{111}In-LiLo-IgM. ^{111}In-DTPA-IgA was washed out of the abscess, while abscess-to-background ratios increased with time [5].

The rapid blood and background clearance of ^{111}In-LiLo-IgM was most probably due to fast, high uptake in liver and spleen. A liver uptake of 5% i.d. g^{-1} in a rat indicates total liver uptake of as much as 60% i.d. Such high physiological liver uptake makes the agent unsuitable for imaging in the upper abdominal area. The much lower liver uptake of ^{111}In-DTPA-IgG is already known to obscure infectious lesions in the liver [13]. In areas outside the vicinity of the liver, liver uptake is irrelevant. Here, the high target-to-background ratios may prove advantageous, for example in the evaluation of infection of the peripheral locomotor system [1, 14]. Similar to the low background activity, the uptake of ^{111}In-LiLo-IgM in the kidneys and lungs is relatively low due to the fast and high liver uptake. Both ^{111}In-LiLo-IgG and ^{111}In-LiLo-IgM showed low bone marrow uptake and insignificant bowel excretion.

In conclusion, chelation with LiLo has a minor influence on abscess targeting of ^{111}In-labelled IgG compared with DTPA conjugation. However, differences in blood clearance and organ uptake do occur. On the basis of experiments in rats, no clear-cut preference for either

method of chelation can be expressed. ^{111}In-LiLo-IgM shows high relative accumulation in abscesses, but also high liver and spleen uptake. The latter renders the ^{111}In-LiLo-IgM preparation unsuitable for infection and inflammation imaging of the upper abdomen, but ^{111}In-LiLo-IgM holds promise for imaging infection outside the trunk region (e.g. the peripheral musculoskeletal system).

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