Is somatostatin receptor scintigraphy suited to detection of acute infectious disease?

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Summary

Fast and accurate delineation of acute infectious foci is very important for adequate management of patients. All currently available scintigraphic techniques require a relatively long timespan between referral to the nuclear medicine department and final diagnosis. Small peptides that bind to receptors on cells in the infectious focus might improve the diagnostic possibilities. Since activated leukocytes express somatostatin receptors, ^{111}In-octreotide, a somastostatin analogue, was tested for its usefulness in detecting acute infection in rats with a calf muscle infection caused by *Staphylococcus aureus*. ^{111}In-octreotide was compared with the much larger protein ^{111}In-labelled human nonspecific immunoglobulin G (^{111}In-IgG). As early as 0.5 h after injection, the ^{111}In-octreotide uptake in the abscess was significantly lower than that of ^{111}In-IgG. Moreover, no ^{111}In-octreotide retention in the abscess over time was noted. In conclusion, somatostatin receptor imaging does not allow scintigraphic detection of an acute infectious lesion. The uptake in an abscess is relatively poor compared to ^{111}In-IgG.

Introduction

Several scintigraphic techniques are currently available for the assessment of the presence and localization of infectious disease. All techniques require a relatively long period of time between referral to the nuclear medicine department and final scintigraphic diagnosis (more than 4 to 6 h). This timespan limits their usefulness in acute infection, especially when immediate surgical intervention is considered. Labelled autologous leukocyte scintigraphy, especially when labelled with ^{99}Tc^m-hydroxymethylpropyleneamine oxime (^{99}Tc^m-HMPAO), may reveal acute infectious lesions within a few hours after administration [1]. However, preparation is rather time-consuming and cumbersome, thereby extending the interval between referral and final diagnosis [2, 3]. The use of radiolabelled proteins such as ^{111}In-labelled human nonspecific immunoglobulin G (^{111}In-IgG) – a large protein which accumulates nonspecifically in infectious foci – reduces the preparation time to approximately 20 min [4–7]. However, even in acute infection final scintigraphic assessment is seldom possible before 18 to 24 h after injection [4, 7]. A novel approach is the use of small chemotactic peptides which bind to receptors in the infectious focus [8]. However, these potentially toxic peptides have not been studied in humans [8]. The use of a labelled somatostatin analogue could be an alternative to chemotactic peptides. Somatostatin receptors are not only expressed on a wide variety of tumour types, but also on activated leukocytes such as macrophages etc., which are of course abundantly present in an acute infectious focus [9, 10]. The radiopharmaceutical can be prepared instantaneously [11]. Extensive clinical testing has revealed no toxic side effects [9, 12, 13]. The aim of this preclinical study was to identify a possible role of a labelled somatostatin analogue, ^{111}In-octreotide, in the early detection of acute infection. For comparison, ^{111}In-IgG was used.

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Methods

Radiopharmaceuticals

The somatostatin analogue ([111In-DTPA-D-Phe[1])-octreotide was obtained commercially (OctreoScan 111, Mallinckrodt Medical B.V., Petten, The Netherlands). Radiolabelling with ^{111}In-chloride was performed according to the manufacturer's protocol. Labelling efficiency was greater than 99%.

Human nonspecific polyclonal IgG (Sandoglobulin, Sandoz AG, Nürnberg, Germany) was conjugated to diethylenetriaminepentaacetic bicyclic anhydride (bicyclic DTPA) according to the method described by Hnatowich *et al.* [14] and labelled with [111]In (Indium chloride, Amersham International Ltd, UK). Labelling efficiency was greater than 95%.

Animal model

Using a previously described method, a calf muscle abscess was induced in young, male randomly bred Wistar rats (weight 220–240 g) after ether anaesthesia with approximately 2×10^8 colony forming units of *Staphylococcus aureus* in 0.1 ml 50:50% suspension of autologous blood and normal saline [15]. The animals were randomly divided into two groups of four rats.

Twenty-four hours after the inoculation of *Staph. aureus* in the muscle, when swelling of the muscle was apparent, 4 MBq of the respective radiopharmaceuticals were injected via the tail vein.

Imaging protocol

After ether anaesthesia, rats were placed prone on the gamma camera and imaged 0.5, 1, 2, 4, 6 and 24 h after injection of either ^{111}In-octreotide or ^{111}In-IgG. The scintigraphic images were recorded with a single-headed gamma camera (Siemens Orbiter, Siemens Inc., Hoffmann Estate, IL) equipped with a parallel-hole, medium-energy collimator and connected to a computer for subsequent data analysis (A[2], Medical Data Systems/ Medtronic, Ann Arbor, MI). Symmetric 20% windows were used for both the 173 and 247 keV energy peaks. Images were obtained with a preset time of 5 min and stored in a 256 × 256 matrix.

The scintigraphic results were analysed by drawing regions of interest over the abscess, over the normal contralateral calf muscle (used as a background region), over the heart (representing blood pool activity) and over the whole animal. Abscess to background ratios, relative activity in the heart (heart to whole body ratio × 100%) and percentage residual activity in the abscess (abscess to whole body ratio × 100%) were calculated.

Statistical analysis

All mean values are given ± 1 standard deviation (S.D.). Statistical analysis was performed using the two-tailed Student's *t*-test.

Results

As shown in Fig. 1 and Table 1, retention in the whole body is significantly higher for ^{111}In-IgG compared to ^{111}In-octreotide 2 h postinjection, indicative of rapid clearance of ^{111}In-octreotide ($P < 0.01$). Rapid clearance from the blood pool is also exemplified by the relative activity in the heart 0.5 h postinjection: only 1.7 ± 0.3% of the whole body activity is seen in the heart region for ^{111}In-octreotide, and 7.6 ± 0.6% for ^{111}In-IgG ($P < 0.01$).
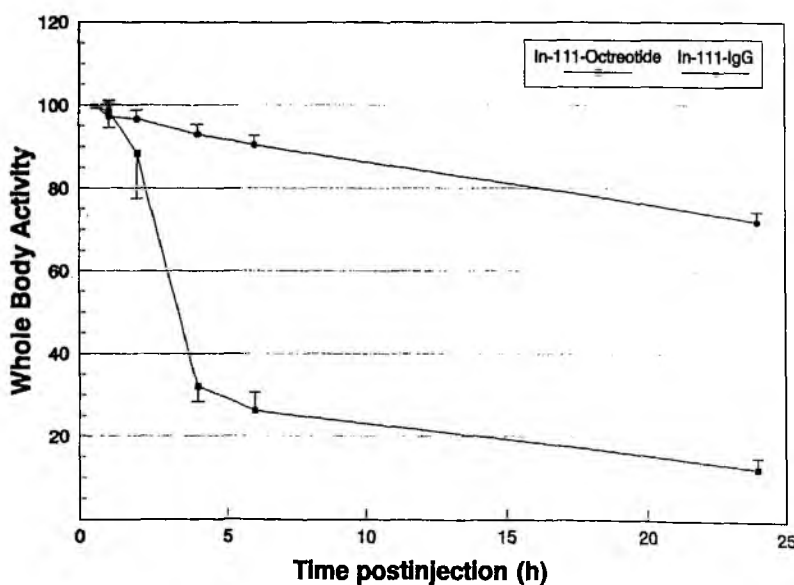

Fig. 1. Whole body retention in rats with calf muscle infection (mean values ± 1 S.D.). For both groups, the activity measured 0.5 h postinjection was set at 100%.

Table 1. Whole body retention in rats with calf muscle infection (mean values ± 1 s.d.). For both groups, the activity measured 0.5 h postinjection was set at 100%*.

Time p.i. (h)	^{111}In-octreotide	^{111}In-IgG	P
0.5	100±0.0	100±0.0	
1	98.4±1.2	97.2±1.5	>0.10
2	88.4±12.3	96.7±0.6	>0.10
4	32.0±4.2	93.0±1.1	<0.01
6	26.1±4.1	90.4±1.0	<0.01
24	12.4±1.5	72.1±1.0	<0.01

*At 0.5 h postinjection, the absolute count rate was not significantly different for both groups.

Table 2. Percentage residual activity in the abscess in rats with calf muscle infection (mean values ± 1 s.d.).

Time p.i. (h)	^{111}In-octreotide	^{111}In-IgG	P
0.5	5.72±1.30	8.18±1.16	<0.05
1	3.91±1.05	9.44±1.28	<0.01
2	2.47±0.94	10.61±1.57	<0.01
4	2.95±1.22	12.03±1.71	<0.01
6	2.63±0.70	14.17±1.61	<0.01
24	2.00±0.44	14.66±1.56	<0.01

One hour after injection, ^{111}In-octreotide activity in the heart region was indistinguishable from activity in the rest of the thorax.

Figure 2 and Table 2 show data on residual activity in the body. As early as 0.5 h postinjection, the percentage residual activity of ^{111}In-IgG is significantly higher than that of ^{111}In-octreotide. Moreover, while ^{111}In-IgG activity in the abscesses shows a relative increase over 24 h, the ^{111}In-octreotide uptake decreases more rapidly than the residual activity in the whole body.

Figure 3 and Table 3 show the abscess to background ratios over time. At 0.5 h postinjection, the ratios are similar for both radiopharmaceuticals. However, no ^{111}In-octreotide retention in the abscess can be noted: ^{111}In-octreotide clears as fast from abscess as from noninfected contralateral muscle, the latter representing background activity. The maximum ^{111}In-octreotide abs-

cess to background ratio in this study just exceeds 2 at one time point. In contrast, ^{111}In-IgG is retained in the abscess. It shows an increasing abscess to background ratio over time which finally reaches 6.08 ± 0.75 at 24 h.

Discussion

The development of an easy to perform imaging technique to detect and localize acute infection within one or at the most a few hours has great potential for patients' management [16]. It may help clinicians to select patients who need immediate invasive therapy and those in whom more conservative management is justified. Labelled leukocyte scintigraphy requires a relatively long preparation time that is responsible for loss of time [1–3]. Using convenient radiopharmaceuticals such as ^{111}In-IgG, a relatively long time is needed before a definite diagnosis can be made [4, 7]. This can be explained by the relatively large size of the protein, implying a relatively slow clearance from blood and soft
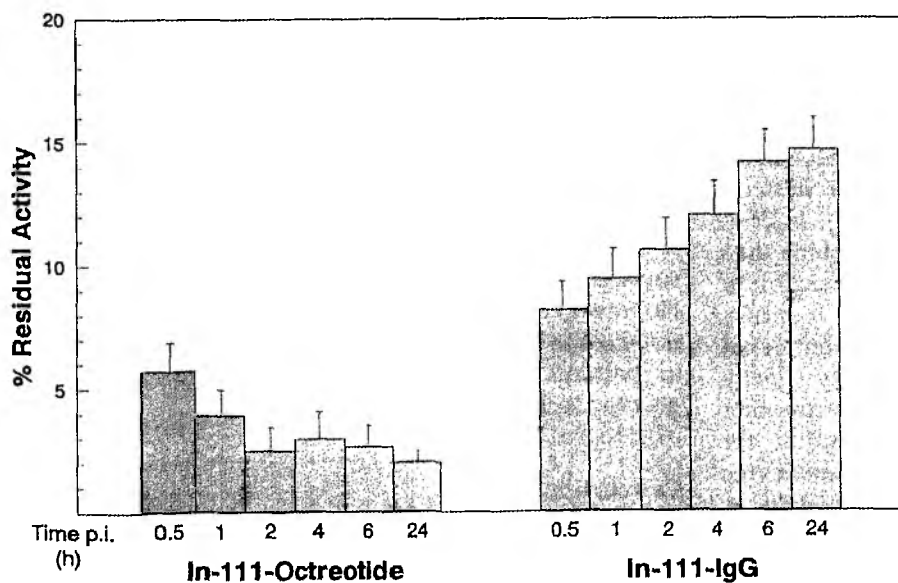
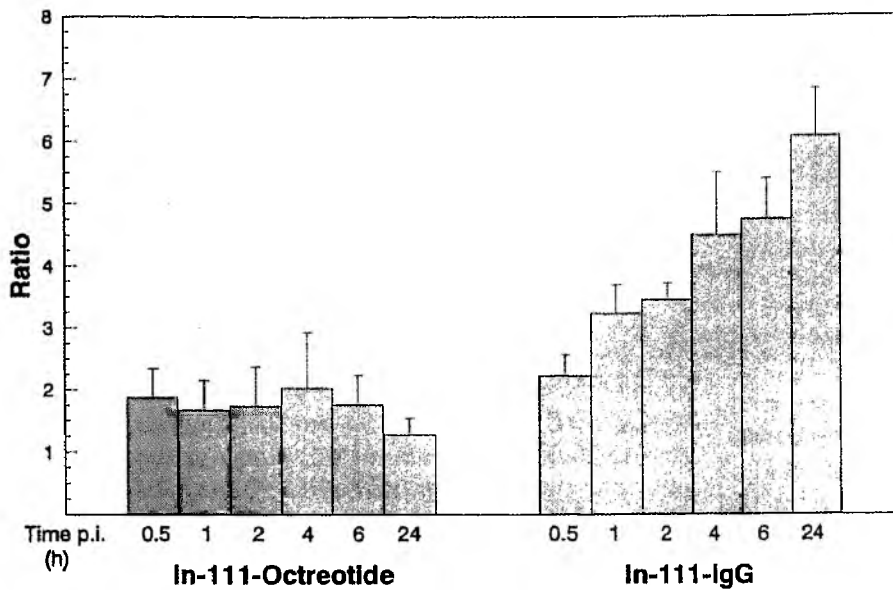**Fig. 2.** Percentage residual activity in the abscess in rats with calf muscle infection (mean values ± 1 s.d.).

Fig. 3. Abscess to background ratio in rats with calf muscle infection (mean values ± 1 S.D.).

Table 3. Abscess to background (contralateral, noninfected calf muscle) ratio in rats with calf muscle infection (mean values ± 1 S.D.).

Time p.i. (h)	^{111}In-octreotide	^{111}In-IgG	P
0.5	1.87±0.49	2.23±0.33	>0.10
1	1.67±0.48	3.23±0.46	<0.01
2	1.74±0.62	3.45±0.27	<0.01
4	2.03±0.90	4.49±1.01	<0.02
6	1.76±0.47	4.75±0.66	<0.01
24	1.28±0.27	6.08±0.75	<0.01

tissues, thus resulting in a relatively slow increase of target to background ratios [15]. For these reasons, the development of smaller molecules with specific receptors in infectious foci seems logical. One possibility is the use of chemotactic peptides [8]. In animal studies, these molecules showed promising results. However, the step from animal to human studies is difficult especially due to bone marrow toxicity [8, 17]. In contrast, ^{111}In-octreotide is a small peptide which has proven to be a safe radiopharmaceutical for detection of various types of tumour in humans [13, 14]. It shows rapid clearance from the blood and subsequent renal excretion, resulting in low background activity shortly after administration [18]. Theoretically, ^{111}In-octreotide could also be an attractive radiopharmaceutical for imaging acute infection, since activated leukocytes express somatostatin receptors [9, 10]. Unfortunately, as demonstrated in the present study, ^{111}In-octreotide shows only modest uptake in acute infectious foci and it

fails to accumulate in the focus with time, thus indicating that specific retention in the focus is absent. This may be caused by the mainly granulocytic infiltration in acute staphylococcal infection, while especially macrophages and activated lymphocytes show high somatostatin-receptor expression. Of course, sufficient high-affinity receptors are necessary to allow retention of such rapidly clearing small peptides in an infectious focus. In contrast, specific receptors are not necessary for accumulation of ^{111}In-IgG, since this radiopharmaceutical is cleared relatively slowly from blood thus allowing a continuous supply of the radiolabelled protein from the blood plasma to the focus, in which the ^{111}In is nonspecifically retained, most probably in a colloidal form [15, 19–21]. As shown previously, early uptake in infectious foci in rats is sufficient for rapid delineation [15]. However, in humans it is rarely possible to make a definite scintigraphic diagnosis using ^{111}In-IgG in acute infectious disease in humans earlier than 4–6 h after injection of the radiopharmaceutical [6, 7].

Although not suited for imaging acute infection, there might be a role for ^{111}In-octreotide scintigraphy in subacute and chronic infection. First reports indicated successful delineation of granulomatous disease [12]. However, the need for rapid scintigraphic results does not exist in this type of infection, so comparative studies in patients are necessary to identify the best scintigraphic technique.

Conclusion

In conclusion, the superiority of ^{111}In-IgG compared to ^{111}In-octreotide in the current animal study indicates

that ^{111}In-octreotide in its present form is not useful for imaging acute bacterial infection due to the absence of sufficient specific accumulation in acute infectious foci. Our results show that there is still a need for development of other small nontoxic peptides with high-affinity binding to receptors on cells that are present in large amounts in acute infectious foci.

Acknowledgements

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