Different behaviour of radioiodinated human recombinant interleukin-1 and its receptor antagonist in an animal model of infection

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Abstract. Recently, we demonstrated that radiolabelled interleukin-1α (IL-1) specifically accumulates in focal infection in mice through interaction with its receptor. Unfortunately, systemic side-effects of IL-1 limit its clinical application. We investigated whether this problem could be circumvented by using the interleukin-1 receptor antagonist (IL-1ra), an equally sized protein that binds to the same receptors as IL-1 without induction of biological effects. Biodistribution of ^{125}I-IL-1 and ^{125}I-IL-1ra was determined in Swiss mice with *Staphylococcus aureus*-induced abscesses in the left calf muscle at 4, 12, 24 and 48 h after injection of either 0.4 MBq ^{125}I-IL-1 or 0.4 MBq ^{125}I-IL-1ra. In vitro, the proteins displayed similar binding characteristics. High-performance liquid chromatographic analysis revealed a tendency for IL-1ra to associate with serum proteins. Both proteins rapidly cleared from most organs. However, the abscess uptake of ^{125}I-IL-1ra was significantly lower than that of ^{125}I-IL-1 at all time points (48 h p.i.: 0.06±0.01%ID/g vs 0.60±0.04%ID/g; $P<0.02$). The abscess-to-contralateral muscle ratios did not exceed 15.5±2.9 for ^{125}I-IL-1ra, while the ratios for ^{125}I-IL-1 reached 46.9±5.7 at 48 h p.i. Despite similar in vitro receptor binding, the abscess uptake of IL-1ra was much lower than that of IL-1. The interaction of IL-1ra with serum proteins in vivo may reduce its availability for receptor binding in the infection. Although on theoretical grounds IL-1ra is very interesting, these characteristics will prevent its development as a clinically useful radiopharmaceutical to image infection.

Key words: Radioiodination – Interleukin-1 receptor antagonist – Infection – Biodistribution

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Introduction

The rather lengthy time required for infection localization with conventional nuclear medicine techniques [1, 2] has stimulated the search for new agents with better imaging properties. Small receptor binding peptides seem to be attractive candidates. Our previous study showed fast background clearance and good accumulation of radiolabelled interleukin-1 (IL-1) – a 17-kDa cytokine – in the infectious focus [3]. Extremely high abscess-to-background ratios were obtained and specific retention at the site of the infection was shown to be due to receptor binding.

Unfortunately, systemic side-effects of IL-1, such as fever and hypotension, hamper its clinical application [4]. These side-effects can occur at doses as low as 10 ng/kg. For these reasons, the potential of the naturally occurring IL-1 receptor antagonist (IL-1ra) was studied. This equally sized molecule is structurally related to IL-1 and binds to the IL-1 receptor with similar high affinity, but does not initiate signal transduction, thus preventing any biological response [5]. Human studies have shown that patients injected with doses up to 10 mg/kg do not show any clinical signs and symptoms or changes in laboratory parameters [6]. Radiolabelled IL-1ra therefore may be a potentially suitable imaging agent that can be applied clinically. In this study the in vitro and in vivo behaviour of radioiodinated IL-1ra was compared with that of IL-1.

Materials and methods

Radiolabelling of IL-1 and IL-1ra

Human recombinant IL-1α (IL-1) and human recombinant IL-1ra were kindly provided by Dr. P.T. Lomedico (Hoffman-La Roche, Nutley, N.J.) and Dr. R.C. Thompson (Synergen, Boulder, Colo.), respectively. IL-1 and IL-1ra were radiolabelled according to the Bolton-Hunter method [7]. Briefly, 40 μg Bolton-Hunter reagent (Pierce, Rockford, Ill.) was radiolabelled with 55 MBq iodine-125

by incubating it with 5 µg *N*-chlorosuccinimide for 10 min at room temperature in methanol. The reaction was terminated by adding 5 µg $Na_2S_2O_5$ to the reaction mixture. The methanol was evaporated with a gentle stream of N_2. Subsequently, 10 µg IL-1 or IL-1ra in 2 µl 1.0 *M* bicarbonate buffer, pH 8.2, was added and incubated for 15 min on ice.

Following the labelling reaction, the reaction mixture was applied to a Sephadex G-75 (Pharmacia, Uppsala, Sweden) column (0.7×50 cm; Biorad, Hercules, Calif.) and eluted with 0.025% gelatine in Tris-HCl buffer 1 m*M* EDTA pH 7.4 to separate labelled proteins from free ^{125}I. The void fractions were pooled and sterilized through a 0.2-µm filter (Millipore, Milford, Mass.). The radiochemical purity of the radiopharmaceuticals was determined as described [3].

Radiolabelled IL-1 and IL-1ra preparations were analysed by high-performance liquid chromatography (HPLC) before and after incubation in mouse serum for 4 h at 37°C in order to test the stability of the radiolabelled proteins. A Shodex protein KW-802.5 packed column with an MW range of 100–50 000 (8×300 mm; Millipore, Milford, Mass.) was used. Tris-HCl buffer with 0.025% gelatine and 1 m*M* EDTA, pH 7.4, was used as the elution buffer. The flow rate was 0.5 ml/min.

Receptor binding assays

The murine cell line EL-4-6.1 [8], a kind gift of Dr. H.R. MacDonald (Ludwig Institute for Cancer Research Epalinges, Switzerland), was cultured at 37°C in a humidified atmosphere of air/CO_2 (95:5) in RPMI 1640 medium (GIBCO, Gaithersburg, Md.) containing 10% foetal calf serum. The EL-4 binding assays and Scatchard analyses were performed as described previously [3].

Competitive binding assay. Live EL-4-6.1 cells were incubated with 2×10^{-10} *M* ^{125}I-IL-1 or ^{125}I-IL-1ra and respectively serially diluted unlabelled IL-1ra or IL-1 (10^{-13}–10^{-6} *M*). After an incubation period of 4 h at 4°C, cell-bound radioactivity was separated from unbound ^{125}I-IL-1 by centrifugation (5 min, 2000 *g*) and measured in the gamma counter.

Biodistribution experiments

Staphylococcus aureus abscesses were induced in the left calf muscles of ether-anaesthetized Swiss mice (20–30 g) as described previously [3]. Twenty-four hours after the inoculation of *S. aureus* in the muscle, when swelling of the muscle was apparent, either 0.4 MBq ^{125}I-IL-1 or 0.4 MBq ^{125}I-IL-1ra was injected in the tail vein. Urine samples were analysed by HPLC 4 h after injection. Groups of five mice were killed under ether anaesthesia by cervical dislocation at 4, 12, 24 and 48 h after injection of the radiopharmaceuticals. Several organs were dissected, weighed and counted in the gamma counter. The measured activity was expressed as a percentage of injected dose per gram tissue (%ID/g).

Analogously, to study the effect of the biological activity of IL-1 on the receptor binding of ^{125}I-IL-1ra in the infectious focus, *S. aureus*-infected mice were injected with a mixture of 0.4 MBq ^{125}I-IL-1ra and 150 ng unlabelled IL-1. The amount of IL-1 was chosen such that biological effects, including a wide range of inflammatory and immunological changes, could be induced [9] without receptor saturation.

Statistical analysis

All values are expressed as mean ± standard error of the mean (s.e.m.). Statistical analysis was performed using the one-way analysis of variance (ANOVA).

Results

Radiolabelling and characterization of the radiolabelled proteins

The labelling efficiencies for both ^{125}I-IL-1 and ^{125}I-IL-1ra were in the range of 20%–30%. The specific activities of ^{125}I-IL-1 and ^{125}I-IL-1ra were 0.3–0.7 MBq/µg. The radiochemical purity of all radiopharmaceuticals was higher than 95% after gel filtration. The preparation of radiolabelled IL-1 remained stable in mouse serum, as shown by the results of HPLC analyses before and 4 h after incubation of ^{125}I-IL-1 in mouse serum at 37°C (Fig. 1A,C). After 4 h, most of the radioiodine was still IL-1 bound. In contrast, the HPLC profiles of ^{125}I-IL-1ra demonstrated a shift of radioactivity from 17 kDa to a higher molecular weight compound (>60 kDa) after 4 h incubation in mouse serum (Fig. 1B,D). To investigate whether this shift could be attributed to either aggregation of IL-1ra or association of ^{125}I-IL-1ra with serum proteins, ^{125}I-IL-1ra was incubated in phosphate-buffered saline (PBS) as well. Samples were analysed after 30 min and 4 h incubation of the protein in PBS or mouse serum at 37°C. The shift to high molecular weight products could already be observed after 30 min in mouse serum. After 4 h, most of the radioactivity was found in the high molecular weight range. However, the shift to the high molecular weight compounds was not found when ^{125}I-IL-1ra was incubated in PBS (data not shown).

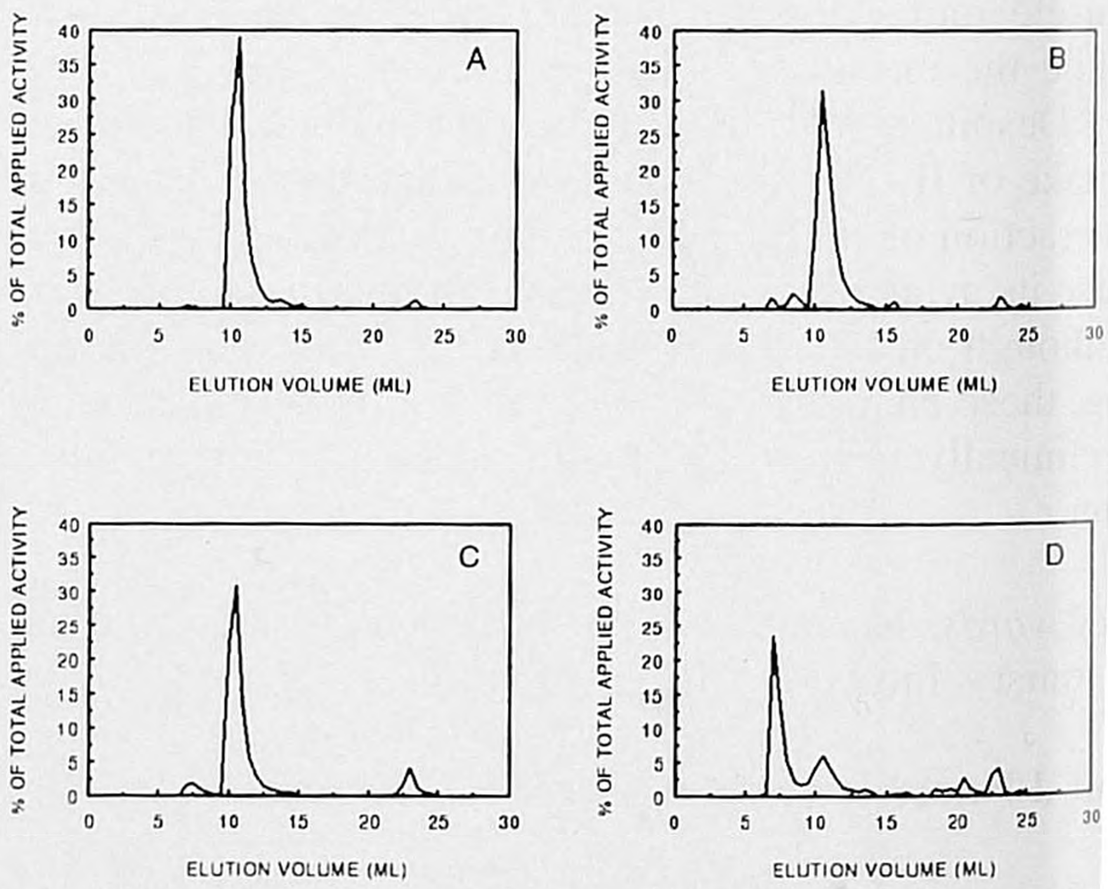

Fig. 1. HPLC profiles of the ^{125}I-IL-1 preparation and the ^{125}I-IL-1ra preparation before injection (**A** and **B**, respectively) and after 4 h incubation in mouse serum at 37°C (**C** and **D**, respectively). The amount of radioactivity collected in each fraction is plotted as a percentage of the total amount added to the HPLC column. The elution volume per fraction was 0.5 ml

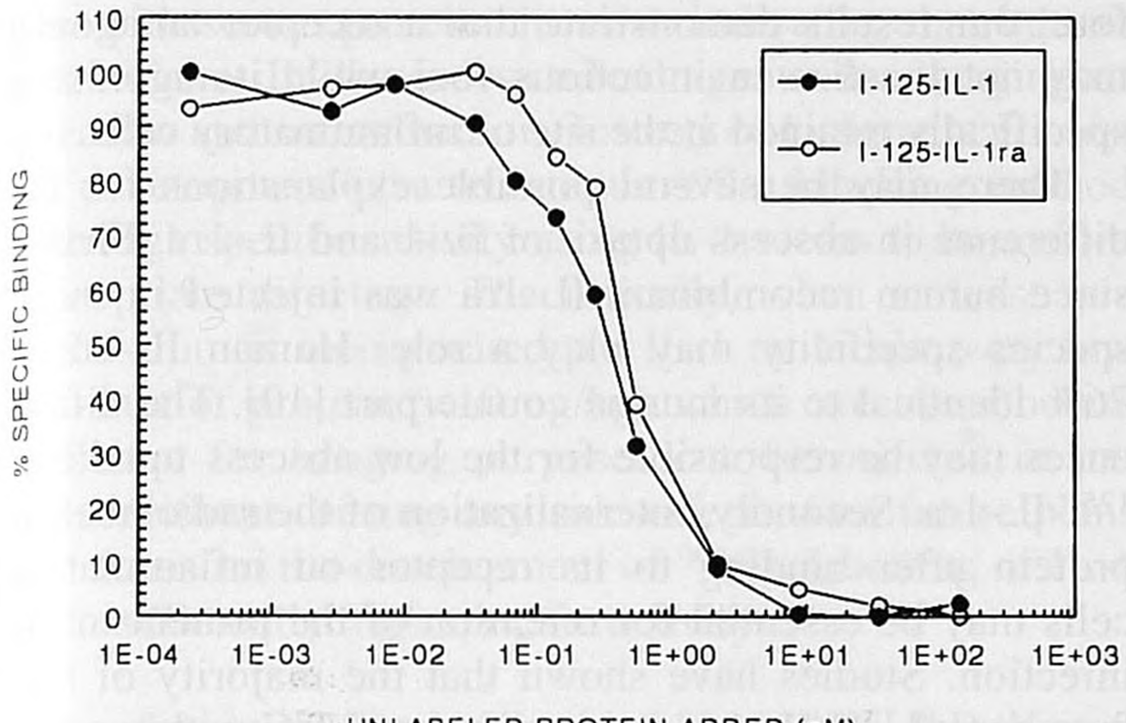

Fig. 2. The competitive binding assay. The binding of ^{125}I-IL-1 to IL-1 receptors on EL-4-6.1 cells was inhibited by increasing amounts of unlabelled IL-1ra. Analogously, the binding of ^{125}I-IL-1ra was inhibited by increasing amounts of unlabelled IL-1

The receptor binding fraction of both ^{125}I-IL-1 and ^{125}I-IL-1ra for conditions representing infinite cell receptor excess was always between 60% and 75%. The non-specific binding was less than 5%. Scatchard analysis revealed that ^{125}I-IL-1 and ^{125}I-IL-1ra bind to the IL-1 receptor on EL-4 cells with almost equal affinities, respectively 5.4×10^{-11} and 4.7×10^{-11} mol/l. Furthermore, as shown by the results of the competitive binding assay (Fig. 2), the binding of ^{125}I-IL-1 to its receptor on the EL-4 cells could be completely inhibited by an excess of unlabelled IL-1ra with an IC_{50} value of 3.5×10^{-10} mol/l. The inhibition of ^{125}I-IL-1ra by unlabelled IL-1 was equally efficient, with an IC_{50} value of 4.6×10^{-10} mol/l.

Biodistribution experiments

Both radiolabelled IL-1 and IL-1ra rapidly cleared from the blood and most other organs (Table 1). After 4 h p.i., only 0.52±0.04%ID/g and 0.98±0.07%ID/g for ^{125}I-IL-1 and ^{125}I-IL-1ra, respectively, was retained in circulation. Most of each of the radiolabelled proteins was excreted via the kidneys during the first hours after injection. Both proteins were mainly excreted in the urine as low molecular weight metabolites: 2.5–3.5 kDa as determined by the Rf values obtained from the HPLC profiles (data not shown).

Despite its rapid blood clearance, ^{125}I-IL-1 was retained in the abscess at >0.6%ID/g from 4 h p.i. onwards. Although ^{125}I-IL-1ra cleared even somewhat slower from circulation, the retention in the abscess was 10 times lower (0.06%ID/g at 48 h p.i.) (Fig. 3). At all time points, the abscess uptake of ^{125}I-IL-1ra was significantly lower than that of ^{125}I-IL-1 ($P<0.02$).

Retention in the abscess of ^{125}I-IL-1, and to a lesser extent of ^{125}I-IL-1ra, was demonstrated by increasing abscess-to-contralateral muscle ratios of both agents in the time course of the study (Fig. 4). The abscess-to-contralateral muscle ratios of ^{125}I-IL-1 increased from 3.8±0.5 at 4 h p.i. to 46.9±5.7 at 48 h p.i. The ratios of ^{125}I-IL-1ra increased to a significantly lower value of 15.5±2.9 at 48 h p.i. ($P<0.005$).

Table 2 summarizes the biodistribution data of *S. aureus*-infected mice i.v. injected with a mixture of ^{125}I-IL-1ra and unlabelled IL-1. The biodistribution data of ^{125}I-IL-1ra in these mice are highly similar to the data shown in Table 1, when no unlabelled IL-1 was adminstered to the mice. The abscess uptake did not change significantly as a result of the co-injection of a small amount of unlabelled IL-1 during the time span of the study.

Table 1. Biodistribution of ^{125}I-IL-1 and ^{125}I-IL-1ra (%dose/g, mean values ±s.e.m.)

Organ	Radiopharmaceutical	4 h p.i.	12 h p.i.	24 h p.i.	48 h p.i.
Blood	^{125}I-IL-1	0.52±0.04	0.09±0.02	0.02±0.002	0.01±0.002
	^{125}I-IL-1ra	0.98±0.07	0.16±0.02	0.03±0.004	0.02±0.0005
Abscess	^{125}I-IL-1	0.98±0.05	0.83±0.07	0.72±0.06	0.60±0.04
	^{125}I-IL-1ra	0.81±0.03	0.23±0.01	0.11±0.01	0.06±0.01
Muscle	^{125}I-IL-1	0.27±0.02	0.08±0.004	0.03±0.004	0.01±0.002
	^{125}I-IL-1ra	0.21±0.02	0.06±0.01	0.01±0.002	0.005±0.002
Thymus	^{125}I-IL-1	0.73±0.11	0.37±0.01	0.22±0.01	0.08±0.01
	^{125}I-IL-1ra	0.52±0.06	0.13±0.006	0.03±0.002	0.01±0.005
Lung	^{125}I-IL-1	1.72±0.23	0.27±0.02	0.09±0.01	0.02±0.004
	^{125}I-IL-1ra	1.08±0.03	0.28±0.006	0.07±0.004	0.03±0.002
Spleen	^{125}I-IL-1	2.42±0.28	0.63±0.05	0.24±0.03	0.11±0.01
	^{125}I-IL-1ra	1.46±0.05	0.39±0.02	0.08±0.01	0.03±0.005
Kidney	^{125}I-IL-1	1.51±0.07	0.59±0.04	0.40±0.03	0.24±0.01
	^{125}I-IL-1ra	6.16±0.78	1.19±0.20	0.44±0.02	0.39±0.04
Liver	^{125}I-IL-1	1.05±0.07	0.32±0.01	0.22±0.01	0.13±0.004
	^{125}I-IL-1ra	3.73±0.51	1.06±0.06	0.10±0.01	0.03±0.007
Intestine	^{125}I-IL-1	1.07±0.09	0.20±0.02	0.07±0.004	0.03±0.004
	^{125}I-IL-1ra	2.39±0.26	0.37±0.06	0.11±0.04	0.06±0.03

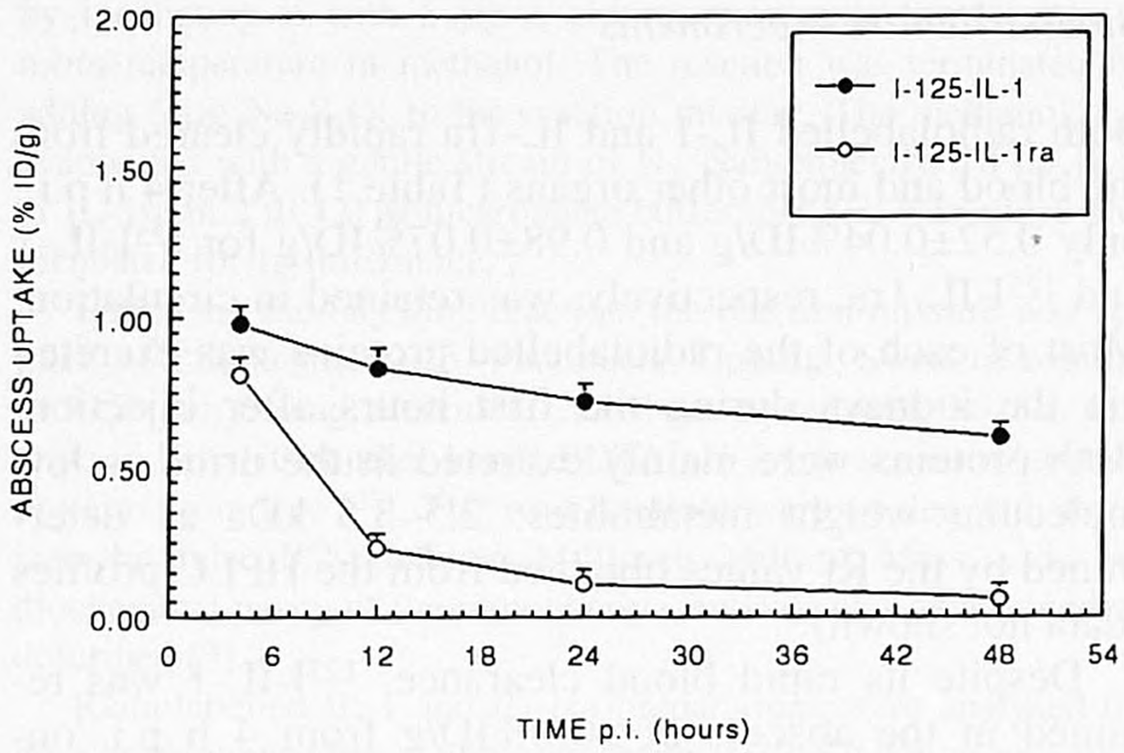

Fig. 3. The absolute abscess uptake of ^{125}I-IL-1 and ^{125}I-IL-1ra at various time points, expressed as %dose/g. The *error bars* indicate s.e.m.

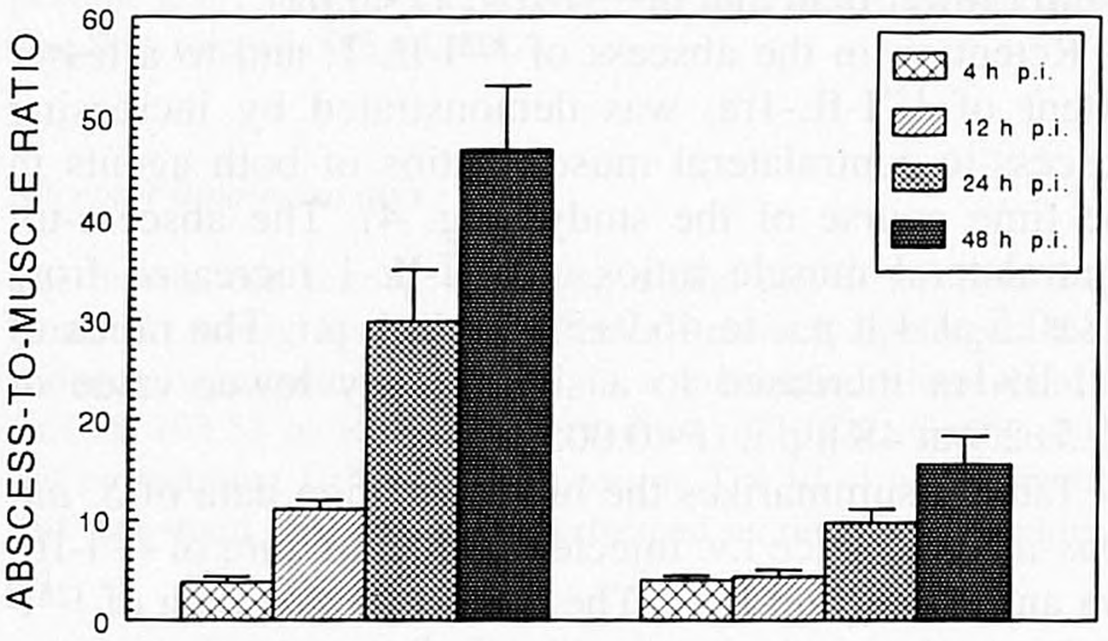

Fig. 4. Abscess-to-(contralateral) muscle ratios of ^{125}I-IL-1 and ^{125}I-IL-1ra at various time points. The *error bars* indicate s.e.m.

Table 2. Biodistribution of ^{125}I-IL-1ra with co-injected unlabelled IL-1 (%dose/g, mean values ±s.e.m.)

Organ	4 h p.i.	12 h p.i.	24 h p.i.	48 h p.i.
Blood	1.02±0.06	0.19±0.02	0.04±0.004	0.01±0.004
Abscess	0.83±0.08	0.26±0.09	0.12±0.02	0.06±0.01
Muscle	0.23±0.04	0.04±0.003	0.01±0.004	0.01±0.004
Thymus	0.72±0.14	0.14±0.03	0.04±0.02	0.01±0.004
Lung	1.04±0.08	0.23±0.01	0.08±0.01	0.03±0.004
Spleen	1.18±0.13	0.25±0.04	0.07±0.01	0.02±0.004
Kidney	5.50±0.39	1.16±0.13	0.53±0.04	0.33±0.03
Liver	3.36±0.42	0.41±0.05	0.09±0.01	0.04±0.01
Intestine	2.61±0.56	0.31±0.13	0.05±0.01	0.02±0.004

Discussion

This paper has described a direct comparison of radioiodinated IL-1 and its receptor antagonist in vitro and in vivo in an animal model of infection. Despite the highly similar in vitro characteristics of the two proteins, the in vivo behaviour was different. The retention of IL-1ra in the abscess was 10 times lower than that of IL-1. Therefore, our results demonstrate that a receptor antagonist may not localize in infectious foci while its agonist is specifically retained at the site of inflammatory cells.

There may be several possible explanations for the difference in abscess uptake of IL-1 and IL-1ra. Firstly, since human recombinant IL-1ra was injected in mice, species specificity may play a role. Human IL-1ra is 76% identical to its murine counterpart [10]. The differences may be responsible for the low abscess uptake of ^{125}I-IL-1ra. Secondly, internalization of the radiolabelled protein after binding to its receptor on inflammatory cells may be essential for retention of the protein in the infection. Studies have shown that the majority of surface-bound ^{125}I-IL-1 is internalized at 37°C within a few hours. It does not undergo degradation for at least 6 h after internalization [11]. However, in contrast to IL-1, surface-bound IL-1ra does not undergo receptor-mediated internalization [5]. Finally, association of IL-1ra with serum proteins in circulation – as demonstrated by HPLC analysis – may make it less available for IL-1 receptor binding in the infectious focus. This phenomenon was not observed for IL-1. Aggregation could be ruled out since most radioactivity was found at 17 kDa after 4 h incubation in PBS at 37°C.

Our study showed that the lack of biological activity of IL-1ra does not explain the low retention in the inflammatory focus. The biological activity of IL-1, including a wide range of inflammatory and immunological effects, did not influence the abscess uptake of IL-1ra when unlabelled IL-1 was co-injected with radiolabelled IL-1ra.

IL-1ra could only be succesfully labelled by using the Bolton-Hunter method. Other radioiodination methods significantly reduced the receptor binding capability of IL-1ra (data not shown). For comparison, IL-1 was also labelled according to the Bolton-Hunter method. We prefer to use the iodogen labelling method for radioiodination of IL-1 because of the higher labelling efficiency [3]. It appeared that the labelling method influenced the clearance of the radiolabelled protein. The Bolton-Hunter method resulted in faster blood clearance of radioiodinated IL-1 than did the iodogen method. Nevertheless the retention in the abscess was similarly high, demonstrating that the binding of radiolabelled IL-1 to inflammatory cells already takes place in the initial hours post injection. During the following hours, non-bound IL-1 will be removed from the tissues, while bound IL-1 is retained in the infection.

Conclusion

IL-1ra could have been the ideal alternative to IL-1 circumvent the toxicity of IL-1 and to use the recept binding capacity for infection imaging. However, th study showed a significantly lower retention in the infe tion of IL-1ra compared to IL-1. This may be caused l specific molecular properties of IL-1ra, such as associ

tion with serum proteins in vivo, indicating that human recombinant IL-1ra is less advantageous for imaging of infection and inflammation. Instead of looking at IL-1ra, the development of a technetium-99m labelling method with high specific activity using IL-1 seems to be a more appropriate direction. Theoretically, when at least one ^{99m}Tc atom is incorporated per IL-1 molecule, very low amounts of protein (<10 ng/kg) which are nevertheless sufficient for imaging purposes can be injected in patients without causing any side-effects. Our future plans will focus on the development of ^{99m}Tc labelling of IL-1 with sufficiently high specific activity.

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