Eur J Nucl Med Mol Imaging (2008) 35:2286–2293
DOI 10.1007/s00259-008-0837-7

ORIGINAL ARTICLE

^{99m}Tc-interleukin-2 scintigraphy in normal subjects and in patients with autoimmune thyroid diseases: a feasibility study

M. Chianelli · S. J. Mather · A. Grossman · R. Sobnak · A. Fritzberg · K. E. Britton · A. Signore

Received: 25 March 2008 / Accepted: 2 May 2008 / Published online: 10 June 2008

Abstract
Purpose Radiolabelled interleukin-2 is a radiopharmaceutical used for the study of chronic inflammatory processes. ^{123}I-labelled interleukin-2 has successfully been used in a large number of patients affected by several immune-mediated diseases. ^{123}I, however, is expensive and not readily available. We have, therefore, developed a method for labelling interleukin-2 with ^{99m}Tc to high specific activity based on the use of an N_3S bifunctional chelating agent. In this paper, we describe the results obtained with ^{99m}Tc-interleukin-2 in a series of eight normal subjects and of 12 patients with autoimmune thyroid diseases.
Methods Biodistribution, pharmacokinetics, haematological and systemic toxicity, radiation absorbed dose and in vivo targeting were studied.
Results Results showed rapid plasma clearance of ^{99m}Tc-interleukin-2 with retention mainly in the kidneys. Biodistribution and kinetics were similar to that observed for ^{123}I-interleukin-2. No acute systemic toxicity was found; a small decrease in peripheral blood lymphocytes was observed in the first hours only in patients, but it was mild and transient. ^{99m}Tc-interleukin-2 accumulated, to varying extents, in the thyroid of all patients affected by autoimmune thyroid diseases but not in the thyroid of normal subjects. The effective dose equivalent of a diagnostic activity of ^{99m}Tc-interleukin-2 (185 MBq) was 1.35 mSv. No correlation was observed between thyroid autoantibodies and uptake of ^{99m}Tc-interleukin-2.
Conclusions The use of ^{99m}Tc-interleukin-2 is safe and simple; the favourable dosimetry and biodistribution and the rapid clearance make it potentially useful for the study of chronic inflammatory diseases such as autoimmune thyroid disease.

Keywords ^{99m}Tc-IL2 · Autoimmune thyroid diseases · Infiltrating lymphocytes · Dosimetry

M. Chianelli (✉)
SSD di Medicina Nucleare, Dipartimento di Diagnostica,
Ospedale Regina Apostolorum,
Via San Francesco, 50,
00041 Albano—Rome, Italy
e-mail: marcochianelli@libero.it

M. Chianelli · A. Signore
Department of Nuclear Medicine and Molecular Imaging,
University Medical Center Groningen, University of Groningen,
Groningen, The Netherlands

S. J. Mather · A. Grossman · R. Sobnak · K. E. Britton
Nuclear Medicine and Endocrinology, St Bartholomew's Hospital,
London, UK

A. Fritzberg
NeoRx Corporation,
Seattle, USA

A. Signore
Nuclear Medicine Unit, S. Andrea Hospital,
2nd Faculty of Medicine, University of Rome "La Sapienza",
Rome, Italy

Introduction

Radiolabelled interleukin-2 (IL2) is a radiopharmaceutical designed for the in vivo study of chronic inflammatory diseases [1]. Previous studies with ^{123}I-labelled IL2 (^{123}I-IL2) in several patients with immune-mediated diseases showed specific targeting of tissues-infiltrated by activated lymphocytes; rapid clearance from the circulation through the kidneys was observed, and major organs of uptake were kidneys, liver and spleen with minimal intestinal excretion. No side effects were reported [2, 3]. Radiolabelled IL2 might be a useful radiopharmaceutical in different immune-

mediated diseases: it might guide the diagnosis and treatment of the disease by providing relevant information about the presence of the ongoing inflammatory process. The efficacy of new, specific, anti-inflammatory drugs could also be tested. It would be necessary, however, to perform studies in an adequate number of patients to evaluate the clinical value of radiolabelled IL2 in different diseases. ^{123}I, however, is expensive and not readily available, and ^{99m}Tc labelling of IL2 would be desirable for larger studies.

We have previously described a technique for ^{99m}Tc labelling of IL2 using a N_3S bifunctional chelating agent with purification by solid phase extraction [4]. The aim of this study was to describe the biodistribution and kinetics of ^{99m}Tc-IL2 in normal subjects, the potential toxic profile and the radiation absorbed dose. We also studied the ability of ^{99m}Tc-IL2 to detect in vivo areas of lymphocytic infiltration in a small number of patients affected by autoimmune thyroid disease.

Materials and methods

Preparation of ^{99m}Tc-IL2

Radiolabelling of IL2 was carried out under aseptic conditions. Interleukin-2 was labelled with ^{99m}Tc using a two-step pre-labelling method as previously described [4] using a bifunctional chelating agent with two functional sites: an N_3S ring, similar to that of MAG3, for the stable coordination of ^{99m}Tc, and an active ester for binding to proteins via the amino groups of lysine residues. Briefly, the ligand was first labelled to high specific activity with ^{99m}Tc (3.7 GBq) in the presence of stannous chloride and gluconic acid at pH 2 at 80°C for 20 min. Radiolabelled ligand was then conjugated to IL2 (100 μg, 1 mg/ml) at pH 9 for 40 min at room temperature. ^{99m}Tc-IL2 was purified by solid phase extraction using a step-gradient elution with acidified ethanol. The radiochemical purity was also evaluated by thin-layer chromatography using ITLC-SGB strips (Gelman) and a scanning radiochromatograph. The strips were developed in acetone for measuring the presence of free ^{99m}Tc. The strips were run in 12% trichloracetic acid (TCA) for measuring technetium colloid formation. Before injection into the patient, ^{99m}Tc-IL2 (0.5 ml in phosphoric acid acidified ethanol) was diluted in 5 ml of a 5% glucose solution containing 0.3% of HSA (final pH 6).

In vivo studies

Informed consent was obtained from all patients and normal subjects studied. Approval for the project was obtained from the local Ethics Committee. Confidentiality of patient identification and data were maintained. Ten minutes before the study, normal subjects and patients received 200 mg of sodium perchlorate i.v. to prevent thyroid and stomach uptake of free ^{99m}Tc possibly generated by the metabolism of ^{99m}Tc-IL2.

Studies in normal volunteers

Eight healthy human volunteers (age 29.3±5 years, six males and two females) were injected with ^{99m}Tc-IL2 (16.1±8.9 μg, 42.6±13.9 MBq). Normal subjects, as requested by the ethics committee and in accordance to the guidance of ICRP62, were injected with a lower activity of ^{99m}Tc-IL2, compared to patients, since in normal subjects the expected level of benefit is minimal and the level of exposure must be in category IIa (effective dose between 0.1 and 1 mSv). Dynamic images were acquired posteriorly for 45 min over the heart, liver, spleen and kidneys; static planar images of the chest and abdomen were obtained at 1, 2 and 4 h. Whole-body and thyroid scans were also acquired at the same time points, and radiation absorbed dose was calculated as described below. Heart rate, body temperature and blood pressure were monitored through the first hour of the study.

Serial blood samples at 5, 10, 15, 25, 35, 45, 60, 75, 120 and 240 min were taken to measure the plasma pharmacokinetics of the tracer, and TCA precipitation of plasma samples was performed. Blood samples taken 1, 3, 24, 72 and 132 h after the injection were used to investigate possible pharmacological effects of interleukin-2 on white blood cell count. Liver function tests, urea and electrolytes were also analysed before and 3 h after the injection. In one subject, a blood sample at 1 h was studied to determine the distribution of radioactivity in the whole blood.

Serial urine samples were taken up to 4 h for calculation of excreted activity and dosimetry calculations. Urine samples were precipitated with TCA for evaluation of ^{99m}Tc-IL2 metabolism.

Studies in patients

Eight patients with Hashimoto thyroiditis, three with Graves' disease and one with primary hypothyroidism were studied. Diagnosis was based on the presence of thyroid autoantibodies (anti-thyroid peroxidase, anti-TSH receptor and anti-thyroglobulin), measurement of hormones (FT3, FT4 and TSH), ^{99m}Tc-pertechnetate thyroid scan and clinical findings. At the time of the study, patients were recently diagnosed and were not receiving anti-thyroid drugs or other medications known to have an effect on lymphocyte activation.

Patients were injected i.v. with 110.2±96.9 MBq of ^{99m}Tc-IL2 (40.2±11.7 μg). After the injection, dynamic

images were acquired posteriorly for 45 min over the heart, liver, spleen and kidney. Static images were acquired at 1 and 3 h over the thyroid. Thyroid uptake of ^{99m}Tc-IL2 was calculated by drawing a rectangular region of interest (ROI) around the organ; thyroid net counts were measured after subtraction of background activity calculated in a second rectangular ROI two pixels larger around the first; absolute thyroid uptake was calculated by converting the thyroid counts in activity according to the counting efficiency of the individual gamma camera. A semi-quantitative measurement of thyroid accumulation of ^{99m}Tc-IL2 was also determined by calculating the thyroid to background ratio by drawing an irregular ROI over the thyroid and a rectangular ROI above the thyroid.

Blood samples were taken before and 10, 20, 40, 60 and 180 min after the injection of the radiopharmaceutical for calculation of blood clearance, TCA precipitation of plasma samples and evaluation of possible pharmacological or toxic effects.

Dosimetry calculations

In normal subjects, a set of anterior and posterior whole-body images, including a standard of known radioactivity, was acquired on a 512×512 matrix at different time points (typically 1, 2, 4 h) after administration of the radiopharmaceutical. Counts determined from the computer images corresponding to regions of interests drawn for the liver, spleen, kidneys, bladder and the standard were converted to activities.

The uptake in each organ was considered to be instantaneous; the clearance curves were obtained from the geometric mean of the anterior and posterior mono-exponential fit to the activity–time curve corresponding to each of the above organs. The activity at time zero and the mean residence time from these clearance curves were then used in conjunction with the MIRD dosimetry system (IBM compatible, MIRDOSE 3, from Oak Ridge Associated Universities, 1994) to calculate the absorbed dose to each organ of interest, as well as to the whole body.

Measurement of urine excretion of ^{99m}Tc-IL2

Urine was collected at 1, 2, 3 and 4 h after the injection of ^{99m}Tc-IL2. For each collection, the total volume excreted was measured, and 1 ml was counted for radioactivity using an automated gamma counter (Ultra-gamma, LKB). The percentage of administered activity excreted in the urine was determined by measuring total excreted radioactivity compared with total activity injected after correction for decay from the time of measurement of the injected activity and for counting efficiency of the gamma counter (71%).

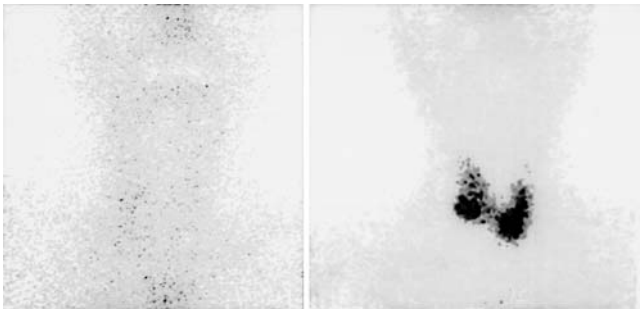

Fig. 1 Anterior view of the neck showing no uptake of ^{99m}Tc-IL2 in the thyroid region of a normal subject (*left*) and in a patient with Hashimoto thyroiditis (*right*)

Results

Preparation of ^{99m}Tc-IL2

The ligand was labelled to high specific activity with a labelling efficiency (LE) of 99% with no colloid formation. IL2 was labelled typically with a LE of 30%; about 30% of IL2, however, was retained by the cartridge, and the final labelling yield, after several passages and 2-h preparation, was about 10%. Starting with 3.7 GBq, the specific activity of ^{99m}Tc-IL2 was about 5.7 MBq/μg. Quality controls showed a radiochemical purity greater than 95% and the absence of pyrogens and of microbial contamination.

Studies in normal volunteers

Studies in normal subjects showed fast plasma clearance. Kidneys were the major organs of accumulation of ^{99m}Tc-IL2; the uptake increased up to 1 h and declined thereafter. Liver and spleen were also detectable. Some excretion in the bowel but no uptake in the thyroid or any other organ was observed (thyroid to background ratio: 1.06±0.05; Fig. 1, Table 1). TCA precipitation of plasma showed that most circulating radioactivity at 1 and 4 h was associated with ^{99m}Tc-IL2 (Fig. 2). TCA precipitation of the urine showed a lower degree of protein-bound radioactivity compared to plasma (Fig. 2). Cumulative urinary excretion of ^{99m}Tc-IL2 4 h p.i. was 26±5.7% of administered activity. Analysis of the distribution of radioactivity within whole blood revealed that 1.3% was associated with the WBC, 10% with the RBC and 88.7% with the plasma.

Table 1 Rate of clearance of ^{99m}Tc-IL2 from different organs and tissues (in minutes)

	Alpha phase	Beta phase
Plasma	5.7±0.2	121±5.8
Kidneys	–	186±31.2
Liver	–	119.4±19.8
Spleen	–	115.2±36

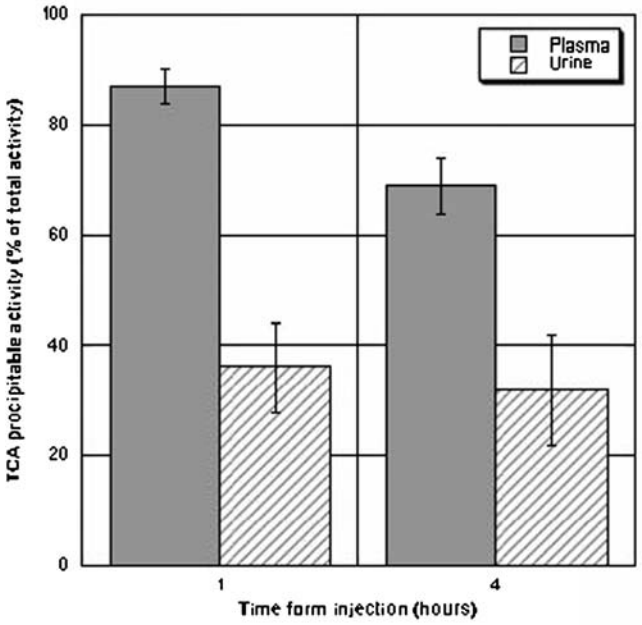

Fig. 2 TCA precipitation of ^{99m}Tc-IL2 in plasma and urine at different time points in normal subjects

No adverse effects from this dose of IL-2 were seen; no changes in heart rate, body temperature and blood pressure were observed; haematological changes were transient and within normal limits (Fig. 3). No changes in platelet counts were observed. The effective dose equivalent (EDE) was calculated to be 7.3 μSv/MBq, i.e. 1.35 mSv for a typical diagnostic scan (185 MBq; Table 2).

Studies in patients

With respect to normal subjects, a higher accumulation of the tracer was observed in the spleen and liver of all patients (Fig. 4). No accumulation in other abdominal organs and minimal intestinal excretion up to 3 h was observed (Fig. 5). TCA precipitable activity of sera at 1 h was mostly protein bound (90.5±5.6%). Haematological changes were transient and within normal limits (Fig. 3). No changes in platelet counts were observed. No side effects were observed.

Studies in patients showed a significant but variable degree of accumulation of ^{99m}Tc-IL2 in the thyroid of all patients with autoimmune thyroid diseases (Table 3, Fig. 6;

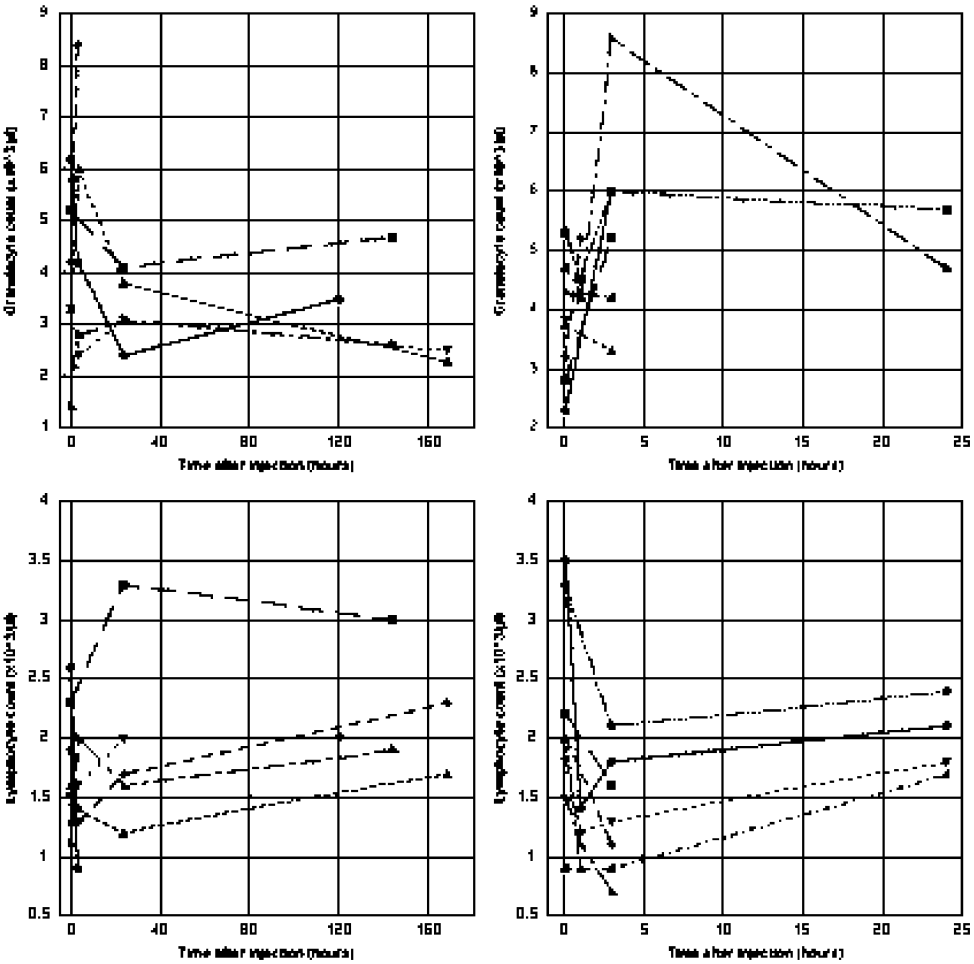

Fig. 3 Effect of ^{99m}Tc-IL2 on granulocytes (*top graphs*), lymphocytes (*bottom graphs*) in normal subjects (on the *left*) and in thyroid patients (on the *right*). All counts are within the normal range

Table 2 Radiation absorbed dose delivered by ^{99m}Tc-IL2

	Values (μGy/MBq)
Kidneys	58.4
Liver	11.3
Spleen	12.7

Effective dose equivalent is 7.3 μSv/MBq; for a typical diagnostic activity of 185 MBq: 1.35 mSv.

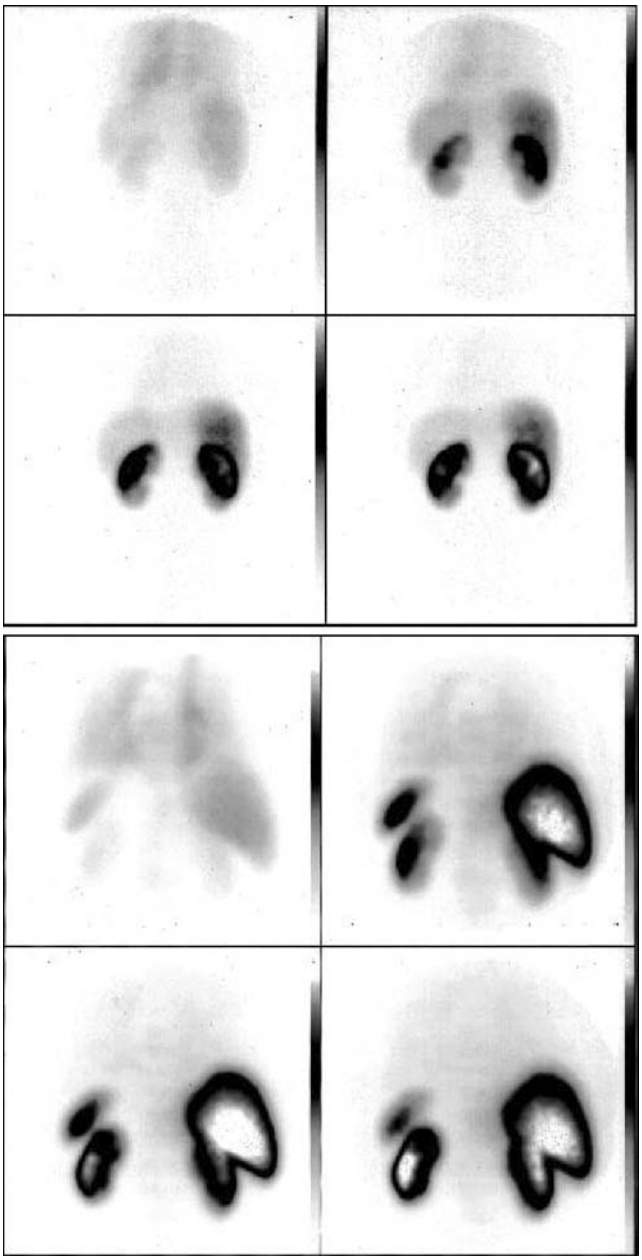

Fig. 4 Posterior views of summed frames of a dynamic study (0 to 45 min) showing early to late frames (*left* to *right*, *top* to *bottom*) in a normal subject (*top*) and in a patient with Hashimoto's thyroiditis (*bottom*)

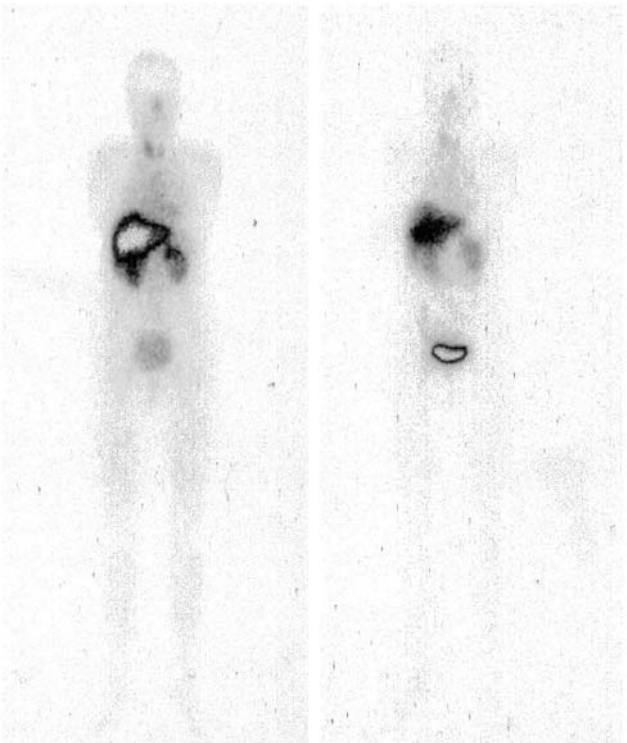

Fig. 5 Anterior (*left*) and posterior (*right*) whole-body scans in a patient with Hashimoto's thyroiditis 3 h after the injection of ^{99m}Tc-IL2

thyroid uptake at 1 h, 0.24±0.09% i.d.; thyroid to background ratio at 1 h, 1.57±0.21; $p<0.0005$ vs normal subjects, Student's t test). Maximum uptake was seen at 1 h; thyroid uptake at 3 h was reduced by half in most patients. No correlation was noted between thyroid accumulation of ^{99m}Tc-IL2 and autoantibody titre.

Discussion

The diagnosis of patients with autoimmune diseases is currently based on the measurements of autoantibodies directed against the target organ [5].

Autoantibodies, however, are indirect markers with uncertain chronological relation with the underlying immune process as they may persist well after the end of the process [6]. They often have poor sensitivity since in a significant number of cases, they are not detectable in peripheral blood [6]. In vivo imaging of chronic inflammatory processes would help to overcome some of these limitations by providing direct evidence of the immune process and might contribute to the management of patients with autoimmune diseases [7, 8].

^{123}I-labelled IL2 (^{123}I-IL2) has been shown to detect with high accuracy lymphocytic infiltration in a series of patients with immune-mediated diseases [2, 3]. Owing to, however, the cost of ^{123}I-IL2, only a limited number of

Table 3 Summary of patient clinical data

Patient no.	Thyroid uptake (% i.d.)	Thy/BKG	Anti-TPO autoantib.	Sex	Age (years)	Diagnosis
1	0.32	1.74	320	F	52	Hashimoto
2	n.a.	1.31	320	F	68	Hashimoto
3	0.28	1.75	1280	F	49	Hashimoto
4	0.14	1.4	3500	F	22	Hashimoto
5	0.23	1.90	1750	F	19	Hashimoto
6	0.23	2.05	1280	M	76	Hashimoto
7	0.05	1.55	640	M	37	Hashimoto
8	0.30	1.85	320	M	70	Hashimoto
9	0.13	1.35	40	F	39	Graves'
10	0.27	1.41	160	F	57	Graves'
11	0.28	1.45	640	F	42	Graves'
12	0.29	1.50	1280	F	52	Prim. hypot.

patients can be studied; ^{123}I, moreover, is not readily available, and the preparation of ^{123}I-IL2 is a lengthy multi-step procedure that requires the availability of a dedicated radiochemistry laboratory. It is, therefore, difficult to organise multi-centre trials to assess the real role of radiolabelled IL2 in a suitable number of patients. The availability of ^{99m}Tc-IL2, preferably in the form of a single-step kit, would greatly simplify the use of this technique.

We have developed a new technique for ^{99m}Tc labelling of IL2 based on the use of an N_3S bifunctional chelating agent with preserved in vitro receptor binding. The technique described in this paper provided high specific activity ^{99m}Tc-IL2 for clinical use. The labelling procedure that was developed is multi-step and lengthy and does not represent a simplification over ^{123}I labelling of IL2. We are working on a simplified labelling technique with the aim to formulate a one- or two-step labelling kit.

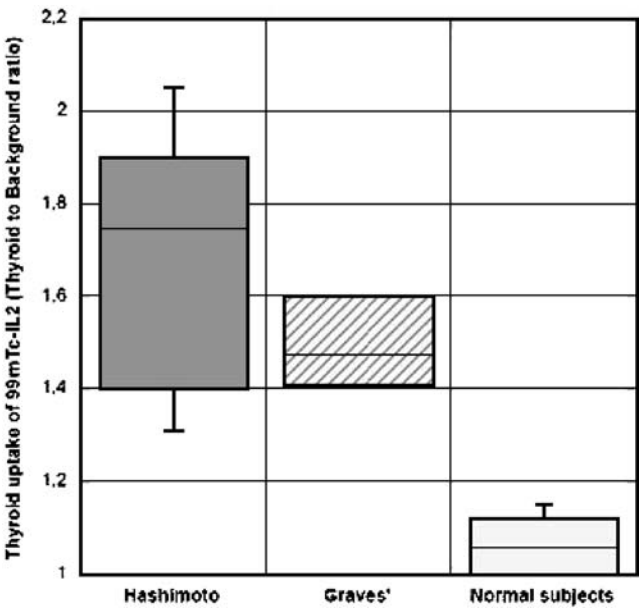

Fig. 6 Thyroid accumulation of ^{99m}Tc-IL2, expressed as thyroid to background ratio in patients with Hashimoto's thyroiditis, Graves' disease (and primary myxoedema) and normal subjects

In this paper, we assess the in vivo use of this new radiopharmaceutical and describe the biodistribution, kinetics, dosimetry, toxic profile and targeting capacity of ^{99m}Tc-IL2 in normal subjects and in patients with autoimmune thyroid diseases.

A low EDE (1.35 mSv for a diagnostic activity equal to 185 MBq) and no toxic effects were observed. This is particularly important in follow-up studies when repeated injections are required, especially in view of the possible use of this radiopharmaceutical in paediatric subjects such as in patients with juvenile diabetes or coeliac disease. Studies in human, healthy volunteers showed high in vivo stability of ^{99m}Tc-IL2, higher than previously shown with ^{123}I-IL2, rapid plasma clearance and low background radioactivity, comparable to that of ^{123}I-IL2. Kidneys were the major organs of accumulation of ^{99m}Tc-IL2, which was excreted in the urine mostly in the form of low molecular weight metabolites, confirming previous studies in animals that demonstrated that IL2 is mainly metabolised in the kidneys [9]. No intestinal excretion was observed.

Compared to normal subjects, studies in patients showed an increased level of accumulation of ^{99m}Tc-IL2 in the liver and, to a lesser extent, in the spleen. More than one mechanism could be advocated for this phenomenon. It is possible that, following the state of immune activation in patients with autoimmune diseases, a greater number of circulating IL2R+ve cells or a greater expression of IL2R is observed in lymphocytes homing into the liver and in the spleen. The binding of ^{99m}Tc-IL2 to soluble IL2R, which has previously accumulated by the liver, is also possible, as well as liver uptake of circulating ^{99m}Tc-IL2/sIL2R complexes. The sensitivity of ^{99m}Tc-IL2 in pathologies of the liver and kidneys may, therefore, be reduced owing to its accumulation in the absence of local pathology. Abbs et al. have, nevertheless, been able to assess kidney graft

rejection by ^{123}I-IL2 in a rat model of kidney transplantation [10].

No thyroid accumulation was seen in normal subjects, whereas in all patients with autoimmune thyroid diseases, a significant thyroid uptake of the tracer was observed, suggesting specific binding to IL2R+ve infiltrating lymphocytes. As a consequence of the rapid uptake of ^{99m}Tc-IL2, image acquisition was completed within 2 h. Before the scan, it was necessary to prepare patients with sodium perchlorate to avoid possible interferences with free pertechnetate released by the metabolism of ^{99m}Tc-IL2. This was well tolerated and did not give any side effect.

Thyroid infiltration by activated lymphocytes has been reported in patients with autoimmune thyroid diseases [11, 12]. Previous studies with ^{111}In-labelled lymphocytes demonstrated accumulation in patients with Hashimoto's thyroiditis but not in patients with Graves' disease [13]. This may depend on the clonal in situ expansion of infiltrating lymphocytes in Graves' disease with no migration from circulating lymphocytes [12]. In the present study, however, ^{99m}Tc-IL2 accumulated in the thyroid of patients with Graves' disease, probably as a consequence of the easy penetration of a small molecule like IL2 in sites of chronic inflammation where the vascular permeability is not greatly increased and access to lymphocytes is more difficult. Thyroid uptake in patients with Hashimoto's thyroiditis was greater compared to that observed in patients with Graves' disease. It is known that both diseases are characterised by lymphocytic infiltration, but in Hashimoto's thyroiditis, the density of thyroid-infiltrating lymphocytes is much higher than in Graves' disease [11, 12]. This is in accordance with our results; a large overlap exists, however, between the degrees of accumulation of the radiotracer in the thyroid of either group (Fig. 6), suggesting that ^{99m}Tc-IL2 can be used for assessing the activity of the disease but not for discriminating between Hashimoto's and Graves' disease.

In Graves' disease patients, a correlation between microsomal autoantibodies and the intensity of the intrathyroidal autoimmune process has been reported [14]. No correlation was noted, however, in this study between thyroid accumulation of ^{99m}Tc-IL2 and autoantibody titre. Although no definitive conclusions can be drawn from the study of this small number of patients, the results obtained so far suggest that there is no direct correlation between humoral-mediated and cell-mediated immunity in autoimmune thyroid diseases. In the three patients with active Graves' disease and exophthalmos, no clear accumulation of ^{99m}Tc-IL2 was noted in the retroorbital space. Lymphocytic infiltration of the retroorbital space has been reported in patients with Graves' disease, and ophthalmopathy and the presence of some cytokines (interferon-γ, IFN-γ, tumour necrosis factor β and interleukin-1α has been reported in the orbital connective tissue of these patients, but the presence of IL2 and of IL2R positive cells is still a matter of debate [15, 16]. It is also possible that a transient, time-related expression of the IL2R occurs and that expression of IL2R might only be observed in the early phases of exophthalmos. This has already been reported for ^{111}In-octreotide, which showed accumulation in the retroorbital space of patients with exophthalmos only during the early phases of the disease [17, 18]. Finally, a sensitivity problem cannot be ruled out due to the limited amount of radioactivity (about 100 MBq) injected in our patients. Indeed, Rendl et al., using ^{123}I-IL2 and injecting much higher activities (about 370 MBq), were able to image the retroorbital infiltration, as well as the pre-tibial myxoedema, better than with ^{111}In-octreoscan in patients with Graves' disease [19]. A controlled study in a larger number of patients is necessary to address this point.

Conclusion

These preliminary results suggest that scintigraphy with ^{99m}Tc-IL2 is simple and safe. Its rapid clearance and favourable dosimetry and biodistribution make it a suitable technique for in vivo imaging of disease activity in patients with autoimmune thyroid diseases and might be used to confirm the immune nature in doubtful cases. Larger studies are in progress in several pathologies to assess the role of ^{99m}Tc-IL2 in the clinical management of autoimmune patients.

Acknowledgements At the time of the study, Marco Chianelli was the recipient of a fellowship of the Juvenile Diabetes Foundation. The staff of the Nuclear Medicine Department at St Bartholomew's Hospital is greatly acknowledged, and in particular Prof. E.A.M. Gale, Dr Vaseem Chengazi and Dr Lorenzo Biassoni for clinical assistance and fruitful discussion. This study has been partially supported by research grants from the Italian Ministry MIUR. The experiments comply with the current laws of the country in which they were performed inclusive of ethics approval.

References

1. Chianelli M, Mather SJ, Martin-Comin J, Signore A. Radiopharmaceuticals for the study of inflammatory processes. A review. Nucl Med Commun 1997;18:437–55.
2. Signore A, Chianelli M, Annovazzi A, Rossi M, Maiuri L, Greco M, Ronga G, et al. Imaging of active lymphocytic infiltration in coeliac disease with ^{123}I-interleukin-2 and its response to diet. Eur J Nucl Med 2000;27:18–24.

3. Signore A, Chianelli M, Annovazzi A, Bonanno E, Spagnoli L, Pozzilli P, Pallone F, Biancone L. ^{123}I-Interleukin-2 scintigraphy for the in vivo assessment of intestinal mononuclear cell infiltration in Crohn's disease. J Nucl Med 2000;41:242–9.
4. Chianelli M, Signore A, Fritzberg AR, Mather SJ. The development of technetium-99m-labelled interleukin-2: a new radiopharmaceutical for the in vivo detection of mononuclear cell infiltrates in immune-mediated diseases. Nucl Med Biol 1997;24:579–86.
5. Dean BM, Becker F, McNally JM, Tarn AC, Schwartz G, Bottazzo GF. Insulin autoantibodies in the prediabetic period: correlation with islet cell antibodies and development of diabetes. Diabetologia 1986;29:339–42.
6. McCulloch DK, Claff LJ, Kahn SE, Shoenfeld SL, Grenbaum CJ, Mauset RS, et al. Subclinical beta-cell dysfunction is not always progressive among first degree relatives of Type 1 diabetes: five years follow-up of the Seattle study. Diabetes 1990;39:549–56.
7. Chianelli M, Parisella MG, D'Alessandra C, Corsetti F, Scopinaro F, Signore A. The developing role of peptide radiopharmaceuticals in the study of chronic inflammation: new techniques for novel therapeutic options. Q J Nucl Med 2003;47:256–69.
8. Signore A, Chianelli M, D'Alessandria C, Annovazzi A. Receptor targeting agents for imaging inflammation/infection: where are we now? Q J Nucl Med Mol Imaging 2006;50:236–42.
9. Ohnishi H, Chao JT, Lin KK, Lee H, Chu TM. Role of the kidney in metabolic change of interleukin-2. Tumour Biol 1989;10: 202–4.
10. Abbs IC, Pratt JR, Dallman MJ, Sacks SH. Analysis of activated T cell infiltrates in rat renal allografts by gamma camera imaging after injection of 123-iodine-interleukin-2. Transplant Immunol 1993;1:45–51.
11. Cohen SB, Weetman AP. Activated interstitial and intraepithelial thyroid lymphocytes in autoimmune thyroid disease. Acta Endocr 1988;119:161–6.
12. Misaki T, Konishi J, Nakashima T, Iida Y, Kasagi K, Endo K, Uchiyama T, Kuma K, Torizuka K. Immunohistological phenotyping of thyroid infiltrating lymphocytes in Graves' disease and Hashimoto's thyroiditis. Clin Exp Immunol 1985;60:104–10.
13. Pozzilli P, Pozzilli C, Pantano P, Negri M, Andreani D, Cudworth AG. Tracking of indium-111-oxine labelled lymphocytes in autoimmune thyroid disease. Clin Endocrinol Oxf 1983;19:111–6.
14. Paschke R, Vogg M, Swillens S, Usadel KH. Correlation of microsomal antibodies with the intensity of the intrathyroidal autoimmune process in Graves' disease. J Clin Endocrinol Metab 1993;77:939–43.
15. Bahn RS, Heufelder AE. Pathogenesis of Graves' ophthalmopathy. N Eng J Med 1993;329:1468–75.
16. Heufelder AE, Bahn RS. Modulation of Graves' orbital fibroblasts proliferation by cytokines and glucocorticoid receptor agonist. Invest Ophthalmol Vis Sci 1994;35:120–7.
17. Postema PT, Krenning EP, Wijngaarde R, Kooy PP, Oei HY, van den Bosch WA, et al. [111In-DTPA-D-Phe1] octreotide scintigraphy in thyroidal and orbital Graves' disease: a parameter for disease activity? J Clin Endocrinol Metab 1994;79:1845–51.
18. Bohuslavizki KH, Oberwörhmann S, Brenner W, Eberhardt J-U, Mönig H, Clausen M, et al. 111In-octreotide imaging in patients with long-standing Graves' ophthalmopathy. Nucl Med Commun 1995;16:912–6.
19. Rendl J, Guthoff R, Schirbel A, Brechtelsbauer D, Schiller D, Seybold S, et al. (Abstract) Iodine-123-interleukin-2 (i-123-IL-2) scintigraphy in Graves' ophthalmopathy (go): a new approach to assess disease activity. Endocr J 2000;47:O–004.