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Research Paper

In SCID Mice with Transplanted Joint Tissues from Rheumatism Patients, a Model Mice of Human Rheumatoid Arthritis, Anti-human Fas Antibody (R-125224) Distributes Specifically to Human Synovium

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Purpose. We investigated the tissue distribution of a humanized anti-human Fas monoclonal antibody, R-125224, in SCID mice transplanted with synovial tissues from patients with rheumatoid arthritis (SCID-HuRAg mice). The binding kinetics of R-125224 was also determined, using isolated human synovial cells.
Materials and Methods. Tissue distribution was assessed at 1, 24 and 168 h after intravenous administration of ^{125}I-R-125224 to SCID-HuRAg mice (0.4 mg/kg). The *in vitro* binding of ^{125}I-R-125224 to isolated human synovial cells was investigated.
Results. After intravenous administration of ^{125}I-R-125224 to SCID-HuRAg mice, the radioactivity distributed to various tissues at 1 h. Thereafter, the radioactivity in the tissues gradually decreased except for the transplanted synovial tissues, in which the radioactivity increased in a time-dependent manner, and at 168 h, the tissue/plasma concentration ratio was about 1. The *in vitro* binding affinity of ^{125}I-R-125224 to human synovial cells was high with a dissociation constant of 1.32 ± 0.62 nM and the binding was inhibited by non-labeled R-125224 in a concentration-dependent manner.
Conclusion. R-125224, a candidate compound for treating rheumatoid arthritis, specifically distributed to the pharmacological target site, human synovium transplanted in SCID mice, with high affinity.

KEY WORDS: anti-Fas antibody; Fas; pharmacokinetics; SCID-HuRAg mouse; tissue distribution.

INTRODUCTION

Fas (CD95) is a major member of the tumor necrosis factor (TNF) receptor superfamily, and is constitutively expressed on various normal and neoplastic cells (1) The binding of Fas with Fas ligand (FasL) induces apoptosis in several types of cells, and Fas-mediated apoptosis plays an important role in the immune system (2–6), as shown in mice with Fas-defective lymphoproliferation and Fas-defective generalized lymphoproliferative disease, which develop severe lymphadenopathy and autoimmune diseases (7,8).

It is known that binding of anti-Fas antibodies to Fas also induces apoptosis in a similar manner as that in the Fas-FasL system and, therefore, the signaling pathway mediated by the Fas-FasL system has been studied using anti-Fas antibody (9). We reported previously that R-125224, a novel humanized anti-human Fas monoclonal antibody, causes Fas-induced apoptosis of activated T lymphocytes and suppresses osteoclastogenesis, indicating that the anti-Fas antibody would have a therapeutic effect on autoimmune diseases such as rheumatoid arthritis (RA) (10). On the other hand, anti-Fas antibody caused apoptosis in hepatocytes, which leads to severe hepatotoxicity, as indicated by the fact that Jo2, a hamster anti-murine Fas antibody, causes hemorrhagic fulminant hepatitis and death in mice *in vivo* (11). This hepatotoxicity of the anti-Fas antibody limits its application to autoimmune disease.

R-125224 originates from HFE7A, a monoclonal mouse IgG anti-Fas antibody, and is produced by grafting the mouse complementarity-determining regions (CDRs) to human IgG. R-125224 has a high affinity to human Fas (13) and shows unique *in vitro* cell selectivity in inducing apoptosis, such as apoptosis induced in activated human lymphocytes but not in human hepatocytes (14). Previously, we prepared a graft versus-host disease model by transplanting splenocytes from human Fas transgenic mice into severe combined immunodeficiency (SCID) mice, and found that administration of

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ABBREVIATIONS: ^{125}I-PBI, *N*-Succinimidyl 4-^{125}I-iodobenzoate; HRPO, horseradish peroxidase; SCID, severe combined immunodeficiency; TMB, 3,3′,5,5′tetramethylbenzidine; TNF, tumor necrosis factor.

HFE7A decreases the level of human Fas-positive lymphocytes by inducing apoptosis in the graft cells without causing liver toxicity, body weight decrease or skin disease (15). These results indicate that R-125224 could be a promising candidate agent for the treatment of RA.

The purpose of the present study is to assess the affinity of R-125224 (anti-human Fas antibody) to human Fas (Fas antigen) *in vitro* and *in vivo* using ^{125}I-labeled R-125224. In the *in vitro* experiment, we examined the binding kinetics of ^{125}I-R-125224 using isolated human synovial cells. In the *in vivo* experiment, we investigated the tissue distribution of ^{125}I-R-125224 in SCID mice transplanted with human synovial tissues (SCID-HuRAg mice, 16–18).

MATERIAL AND METHODS

Reagents

R-125224 and Fas-AIC2, which is a Fas antigen for assay of R-125224 by ELISA, were produced in Sankyo Co., Ltd. (Tokyo, Japan). A protein radio-labeling reagent, *N*-Succinimidyl 4-^{125}I-iodobenzoate (^{125}I-PBI) was purchased from PerkinElmer Life Sciences Inc. (Wellesley, MA, USA). An enzyme-labeled antibody, anti-human IgG conjugated with horseradish peroxidase (HRPO), was purchased from Amersham Biosciences (Buckinghamshire, UK). Collagenase was purchased from Sigma–Aldrich Japan Inc., Tokyo, Japan).

Human Synovial Tissues

Fresh human synovial tissues were obtained from patients with rheumatoid arthritis undergoing arthroplasty or synovectomy at Toyama Medical and Pharmaceutical University and from Tohoku Rosai Hospital. Written informed consent was obtained from all patients.

^{125}I-Labeling of R-125224

An aliquot of ^{125}I-PBI reagent for ^{125}I-labeling of proteins was dried by a nitrogen gas stream. An aliquot (0.5 ml) of a solution containing 2 mg/ml R-125224 adjusted to around pH 8.5–9 by 0.1N Na_2CO_3 was added to the dried ^{125}I-PBI reagent and the mixture was mixed well. After 30 min reaction at room temperature, 50 μl of 2 mM glycine solution was added to the mixture to terminate the reaction. The mixture was applied to a PD-10 column which had been pre-conditioned with 20 ml of phosphate buffered saline (PBS) and eluted by PBS. Ten drops of the eluate were collected for each fraction (around 0.5 ml). The radioactivity of each fraction was measured by a gamma counter. The first peak of radioactivity was identified as the intact ^{125}I-labeled R-125224 fraction. The fractions constituting the first radioactive peak were combined, and used as the stock solution of ^{125}I-R-125224. The protein concentration of ^{125}I-R-125224 stock solution was 0.81 mg/ml.

Binding of ^{125}I-R-125224 to Human Synovial Cells

The synovial tissues collected from three patients were separately minced with scissors and each digested with 0.5 mg/ml collagenase for 1 to 2 h at 37°C in DEME. After the digested samples were allowed to stand for 5 min at room temperature to precipitate large debris, the supernatant was centrifuged and suspended in Ham's F12 medium supplemented with 50 U/ml of penicillin, 50 μg/ml of streptomycin, 2.5 μg/ml Fungizone® (Invitrogen Corporation, Carlsbad, CA, USA) and 10% fetal bovine serum (F10F). The synoviocytes were cultured in F10F medium in a cell culture dish (Lot No. 430599, Becton Dickinson and Company, Franklin Lakes, NJ, USA). When 70 to 90% of confluence was reached, the synoviocytes were treated with trypsin-EDTA, suspended in a storage buffer, transferred into a cell banker and stored in a freezer at −80°C. For the binding assay, the synoviocytes were thawed according to a conventional procedure and maintained in F10F medium in a cell culture flask.

Human synovial cells prepared at 2×10^6 cells/ml were kept on ice before the experiments. Ten microliters of ^{125}I-R-125224 solutions (final: 100–1,000 ng/ml) and 100 μl of RPMI 1640 medium containing 1% bovine serum albumin (BSA) were added to 390 μl of the cell suspension and the mixture was incubated on ice for 1 h. A 200-μl aliquot of the mixture was taken in duplicate (total sampling amount: 400 μl) and each aliquot was transferred into a tube containing 100 μl of mixed oil consisting of 20% olive oil (Wako Pure Chemical Industries Ltd., Osaka, Japan) and 80% dibutylphthalate (Wako Pure Chemical Industries Ltd.). Each tube was centrifuged at 15000 rpm for 5 sec (Microfuge E, Beckman Co., Fullerton, CA, USA) to precipitate the cells through the oil layer. The tubes were stored at −80°C for more than 30 min to freeze the content. The frozen supernatants and sediment were separately collected by cutting with a tube cutter. The supernatants and sediment placed in the counting vials were subjected to radioactivity measurement by a gamma counter (RIASTAR, Packard Instrument Company, Wellesley, MA, USA). The gamma counting was conducted for 1 min for each sample. The radioactivity background was obtained by counting the vials containing only the medium. The experiments were conducted in duplicate for each lot of synovial cells ($N=3$).

For examining the inhibitory effects of non-labeled R-125224, incubation of the human synovial cells with ^{125}I-R-125224 (final: 400 ng/ml) was conducted according to the same procedure described above except for adding 100 μl of the solution of non-labeled R-125224 (final: 0.08–80 μg/ml) containing 1% BSA. The concentration of radioactivity in the cell-free fractions and cell sediment fractions were measured by the same method as above.

The dissociation constant (K_d) and maximum binding ($Bound_{max}$) were calculated by Scatchard plots according to the following equation:

$$[\text{Bound}]/[\text{Free}] = -1/K_d \times [\text{Bound}] + \text{Bound}_{max}/K_d$$

where, [Bound]/[*Free*] and [Bound] represent the bound/free ratio and net cell-bound concentration, respectively. [Bound] was calculated by subtracting non-specific binding from the total binding. Non-specific bound-concentration defined as the residual bound-concentration in the presence of 200-fold of non-labeled R-125224. The K_i value of non-labeled R-125224 was estimated by fitting the binding data of ^{125}I-R-125224 in the presence of non-labeled R-125224 to the

following equation using WinNonlin™ professional Edition (Version 3.1, Pharsight Co., Mountain View, CA, USA).

$$\% \text{ control binding} = \left[(K_d + [^{125}\text{I-R-125224}])/\left([^{125}\text{I-R-125224}] + K_d \times (1 + [\text{non-labeled R-125224}]/K_i)\right)\right] \times 100,$$

where [^{125}I-R-125224] and [non-labeled R-125224] represent the concentrations of ^{125}I-R-125224 and non-labeled R-125224, respectively.

The inhibition constant (K_i) value was calculated using the data obtained from the isolated synovial cells from one individual, because synovial cells from the rest of two patients were not sufficient for determination of K_i.

Affinity of R-125224 to Fas Antigens

A 96-well plate (Lot No. 430341, C96 Maxisorp™, Nalge Nunc International, Rochester, NY, USA) was coated with 100 μl/well of Fas-AIC2 (Human Fas antigen for ELISA) solution diluted with 0.05 M carbonate-bicarbonate buffer (pH 9.6), which corresponded to the Fas-AIC2 concentration of 0.704 μg/ml. After the plate was allowed to stand for 1 h at 37°C, the liquid was removed by suction from the wells, which were subsequently filled with blocking buffer (distilled water containing 50% Block Ace™, Dainippon Pharma Co., Ltd, Suita, Japan) and kept at 37°C for 1 h. The wells were emptied and washed three times each with 300 μl of PBS containing 0.5% Tween 20 (washing buffer). R-125224 solution and Fas antigen, either the human Fas antigen or mouse Fas antigen, solution were mixed. The final concentration of R-125224 solution was 10 ng/ml and those of the antigen solutions were 0, 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1,000, 3,000 and 10,000 ng/ml. After addition of the mixture of R-125224 and the Fas antigen mixture to the wells, the plate was incubated at 37°C for 1 h. Then the wells were washed in the same manner as described above, 100 μl of anti-human IgG with HRPO was added to the wells and the plate was incubated at 37°C for 1 h. After washing the wells, 100 μl of 3,3′,5,5′-tetramethylbenzidine (TMB) soluble reagent was added as a substrate of HRPO and the plate was incubated at room temperature for 8 min. Finally, 100 μl of TMB stop buffer was added to each well and the absorbance was read at 450 nm.

Preparation of SCID-HuRAg Mice

The synovial tissues excised from the patients by surgery were used for implants in SCID mice and the size of each specimen was adjusted to a block of 5–10 mm in diameter. The SCID mice at 7 weeks of age (Charles River Japan, Inc, Yokohama, Japan) were anesthetized with diethyl ether and one block of the synovial tissue was transplanted onto the back of each mouse.

The whole surgical procedure was performed under aseptic conditions. Nine SCID-HuRAg mice were administered ^{125}I-R-125224 intravenously at 3 weeks after xenografting. Animals were maintained under conventional housing conditions during the acclimatization and the experiment. All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals in Sankyo Co., Ltd.

Tissue Distribution Study

The stock solution of ^{125}I-R-125224 was diluted with saline to prepare a dosing solution containing 0.214 mg protein/ml of ^{125}I-R-125224 (30.1 MBq/mg) at dosing day. The protein concentration of the dosing solution was adjusted so that 0.05 ml was given to each SCID-HuRAg mouse (average body weight: 26.79 g) at a dose of 0.4 mg/kg.

The dosing solution of ^{125}I-R-125224 was administered to nine mice intravenously via tail veins. After intravenous administration of ^{125}I-R-125224, blood samples were taken by cardiac puncture under diethyl ether anesthesia at 1, 24 and 168 h post-dose to prepare plasma samples. Subsequently, the liver, kidney, pancreas, heart, lung, brain, white adipose tissue, testis, muscle, spleen, adrenal grand, brown adipose tissue, stomach, synovial tissue, thyroid, infra-axillary lymph node, small intestine and large intestine were excised from the mouse carcass. The tissues and organs extirpated were weighed in their wet condition. The samples prepared in the vials were subjected to radioactivity measurement by gamma counting. Gamma counting was conducted for 1 min for each sample.

To determine the stability and biodegradation of ^{125}I-R-125224 in the plasma, liver and synovial tissues, each sample was added to Laemmli sample buffer (Nippon BioRad Laboratories, Tokyo, Japan), and the mixture was subjected to sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) on a 5–20% Tris-glycine gel (Nippon Bio-Rad Laboratories).

RESULTS

Kinetics of Binding of ^{125}I-R-125224 to Human Synovial Cells

The kinetics of binding of ^{125}I-R-125224 to human synovial cells was investigated after incubation for 1 h. In a preliminary experiment using the synovial cells from one donor, binding of ^{125}I-R-125224 reached the maximum value at 30 min after starting the incubation (data not shown). The Scatchard plots for binding of ^{125}I-R-125224 to human synovial cells are shown in Fig. 1. The K_d and Bound_{max} values calculated are listed in Table I and were 1.32 ± 0.62 nM and 0.129 ± 0.064 pmol/10^6 cells, respectively. A high binding affinity of R-125224 as indicated by a low dissociation constant at a nanomolar order shows that ^{125}I-R-125224 tightly bound to the human synovial cells. The competition binding assay shown in Fig. 2 demonstrated that the binding of ^{125}I-R-125224 was inhibited by non-labeled R-125224 in a concentration-dependent manner. The excess of control human IgG added to the incubation mixture did not inhibit the binding of ^{125}I-R-125224 to human synovial cells (data not shown). The K_i value of non-labeled R-125224 for inhibiting the binding of ^{125}I-R-125224 to human synovial

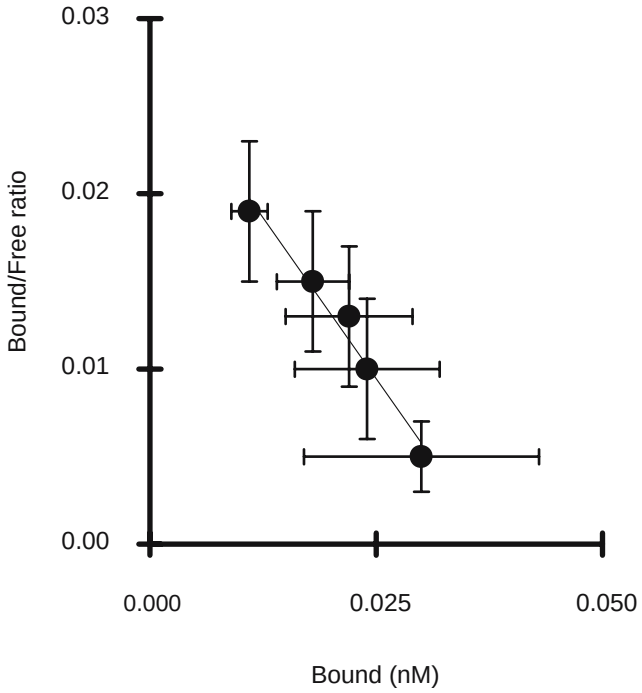

Fig. 1. Scatchard plots for binding of ^{125}I-R-125224 to human synovial cells. Results are expressed as mean ± SD of three lots.

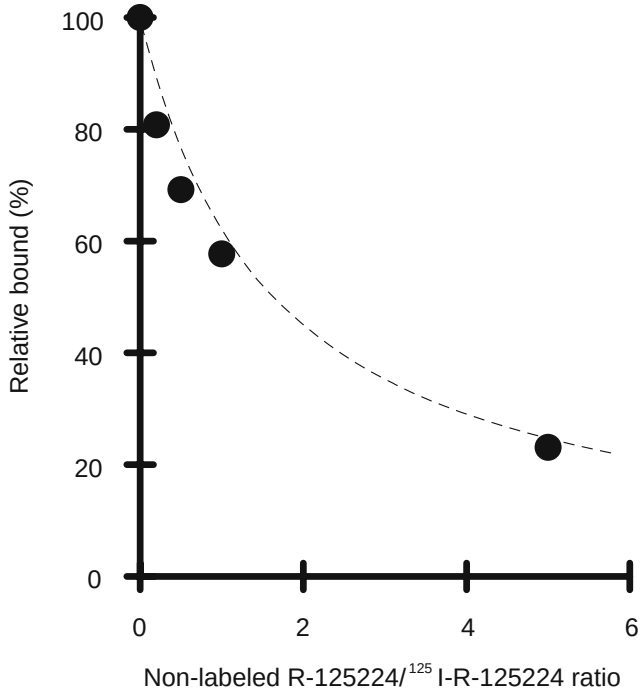

Fig. 2. Inhibition of binding of ^{125}I-R-125224 to human synovial cells by non-labeled R-125224.

cells collected from one individual was 1.50 nM, which was consistent with the K_d value of ^{125}I-R-125224 (1.32 nM), indicating that the binding affinity of ^{125}I-R-125224 was comparable to non-labeled R-125224.

Affinity of R-125224 to Fas Antigens

Binding of R-125224 to the solid-phase of human Fas antigen (Fas AIC2), in the presence of human Fas antigen or mouse Fas antigen, was measured by ELISA to compare the affinity of R-125224 to human Fas with that to mouse Fas.

Absorbance at 450 nm (% of control) plotted against the ratio of antigen concentration to R-125224 concentration is shown in Fig. 3. The absorbance ratio in the presence of mouse Fas was almost 100% in the concentration range tested, demonstrating no affinity of R-125224 to mouse Fas. On the other hand, the absorbance ratio in the presence of human Fas dramatically decreased with increase in the antigen/R-125224 ratio and was less than 10% of the control value at the ratio of 10 or more. These results indicated that R-125224 binds specifically to human Fas, not to mouse Fas antigen.

Table I. Binding Study of ^{125}I-R-125224 to Human Synovial Cells and Human Lymphocyte

	K_d (nM)	$Bound_{max}$ (pmol/10^6 cells)
Human synovial cells	1.32 ± 0.62	0.129 ± 0.064
Human lymphocytes[a]	4.27 ± 1.46	0.050 ± 0.011

[a]The data of human lymphocyte was quated from Nakayama *et al.* (14).

Tissue Distribution of ^{125}I-R-125224 in SCID-HuRAg Mice

Concentrations of the radioactivity after intravenous administration of ^{125}I-R-125224 to SCID-HuRAg mice at a dose of 0.4 mg/kg are shown in Fig. 4. At 1, 24, and

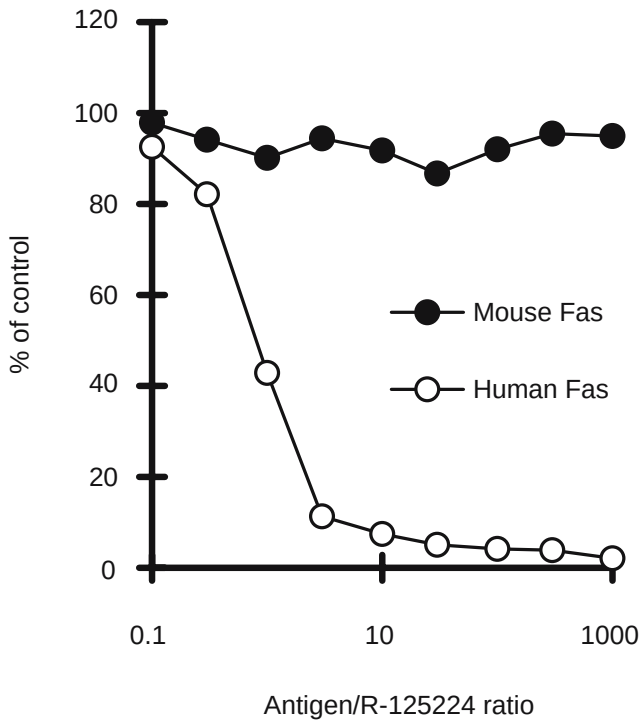

Fig. 3. Binding affinity of R-125224 to mouse and human Fas antigen.

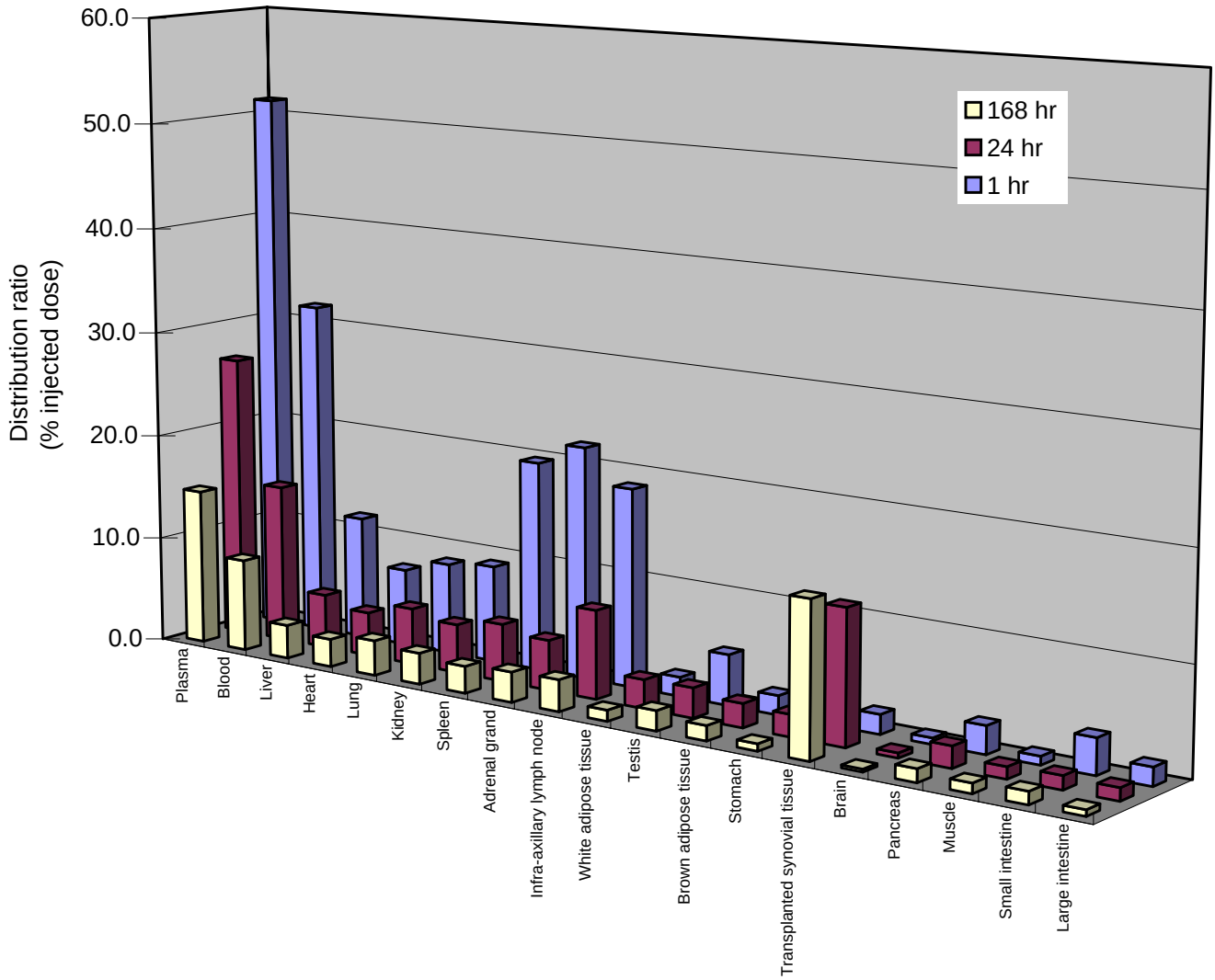

Fig. 4. Tissue distribution ratio after intravenous administration of ^{125}I-R-125224 to SCID-HuRAg mice at a dose of 0.4 mg/kg. Results are expressed as mean of three mice.

168 h post-dose of ^{125}I-R-125224, concentrations of the total radioactivity in the plasma were 5.51 ± 0.50, 2.87 ± 0.46 and 1.57 ± 0.47 μg eq./ml, respectively. In tissues and organs, concentration of the radioactivity was the highest in the thyroid (data not shown). The accumulation in the thyroid was considered to be distribution of free ^{125}I released from ^{125}I-R-125224 and was 0.02% of the dose at 1 h post-dose, 0.08% of the dose at 24 h post-dose, and 0.21% of the dose at 168 h post-dose. At 1 h post-dose, as for the tissues other than the thyroid, high radioactivity concentrations were observed in the liver, spleen, adrenal grand and lymph node, and these were gradually decreased thereafter with time. On the other hand, the concentrations of the radioactivity in transplanted synovial tissues gradually increased in a time-dependent manner and were 0.195 ± 0.155, 1.34 ± 0.32 and 1.53 ± 0.32 μg eq./ml at 1, 24 and 168 h post-dose, respectively. At 168 h post-dosing, it was the highest among all tissues and organs except for the thyroid.

As shown in Fig. 5, the tissue/plasma concentration ratios (K_p) for the tissues and organs were almost constant through 1 to 168 h post-dose in all tissues and organs except for transplanted synovial tissue, indicating that the radioactivity as R-125224 or R-125224-related substances is eliminated from these tissues in parallel to the plasma and does not accumulate in the tissues and organs.

The K_p values of the liver were 0.228 ± 0.041, 0.190 ± 0.040 and 0.207 ± 0.050 at 1, 24 and 168 h post-dose, respectively. On the other hand, in the transplanted synovial tissue, the K_p values increased gradually and were 0.034 ± 0.026, 0.481 ± 0.169 and 0.990 ± 0.121 at 1, 24 and 168 h post-dose, respectively, indicating that ^{125}I-R-125224 is distributed to transplanted synovial tissue specifically.

Stability and Biodegradation of R-125224 Analyzed by SDS-PAGE

The plasma, liver homogenate and synovial tissue samples obtained from the tissue distribution study described above, were analyzed by SDS-PAGE in combination with autoradiography (Fig. 6) and the residual percentage of the intact

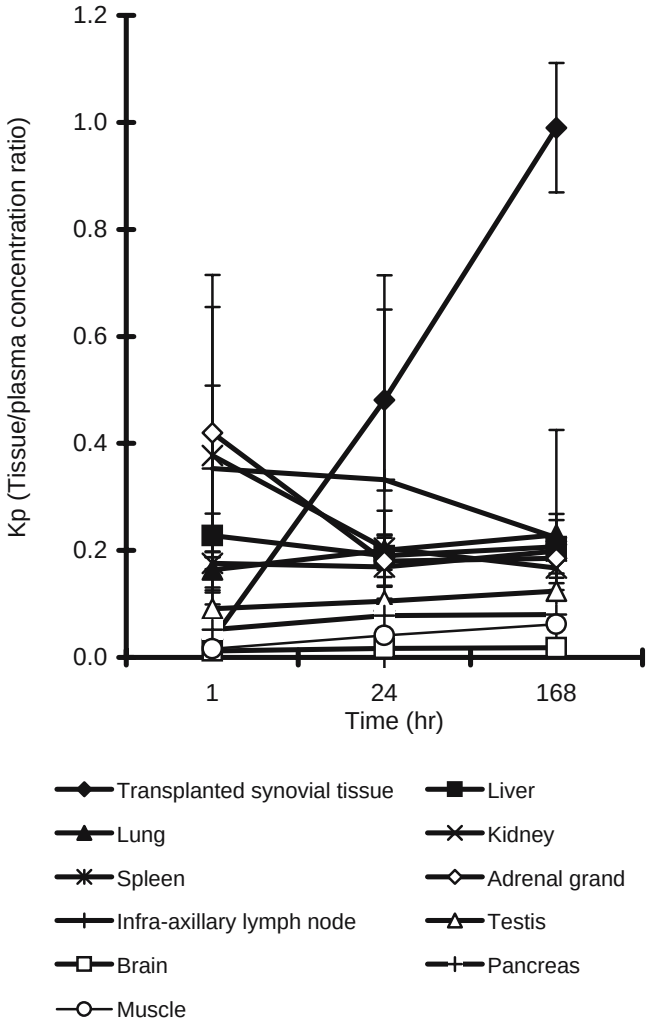

Fig. 5. Tissue/plasma concentration ratio (K_p) after intravenous administration of ^{125}I-R-125224 to SCID-HuRAg mice at the dose of 0.4 mg/kg. Results are expressed as mean ± SD of three mice.

form of ^{125}I-R-125224 in the total radioactivity is shown in Fig. 7. The SDS-PAGE autoradiograms demonstrated that more than 60% of the radioactivity in synovial tissue existed as the intact form of ^{125}I-R-125224 up to 168 h. In the liver, most of R-125224 was degraded to the fragments with lower molecular weights and only a small amount of the intact form was detected in the liver. Constantly about 90% of radioactivity in the plasma existed as the intact form over a period up to 168 h post-dose, indicating negligible degradation in the plasma.

DISCUSSION

The aim of the present study was to assess the affinity of R-125224 to human Fas-antigen *in vitro* and *in vivo* using ^{125}I-labeled R-125224. We have shown that ^{125}I-R-125224 binds to human synovial cells with high affinity *in vitro* and is specifically distributed *in vivo* to the human synovial tissues transplanted into SCID-HuRAg mice.

Nakayama *et al.* (14) examined the apoptosis mediated by the R-125224 and Fas system using the human lymphoid

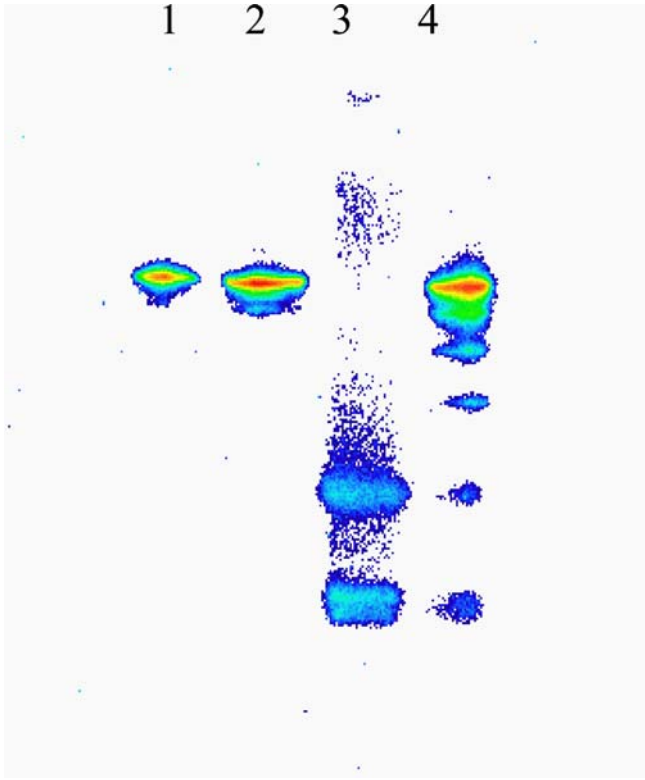

Fig. 6. SDS-PAGE autoradiogram of dosing solution, plasma, liver and synovial tissue. *Lane 1*: dosing solution, *lane 2*: plasma at 168 h, *lane 3*: liver at 168 h and *lane 4*: synovial tissue at 168 h.

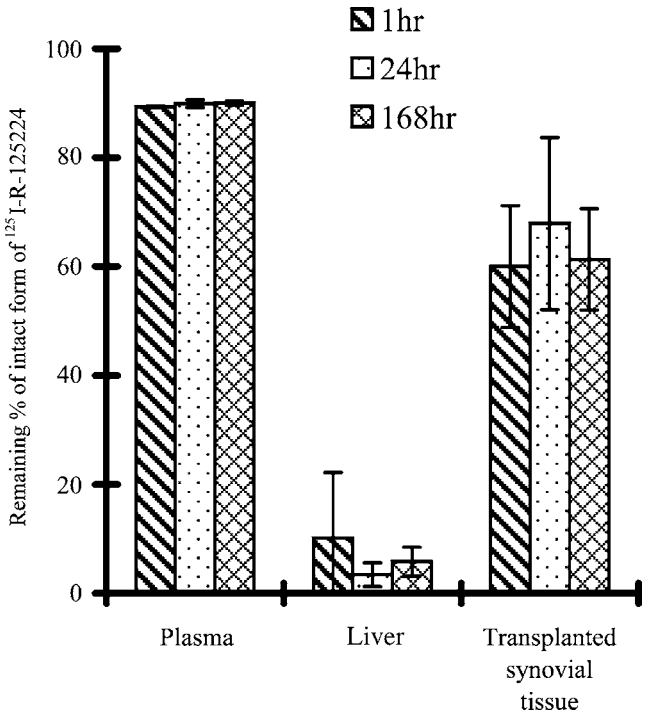

Fig. 7. Remaining percent of intact form of ^{125}I-R-125224 in plasma, liver and transplanted synovial tissue at 168 h post-dose in autoradiogram of SDS-PAGE.

cell lines (H9, SKW6. 4, HPB-ALL and Jurkat cells), activated lymphocytes and human hepatocytes, and reported that R-125224 induces apoptosis in H9 and SKW6.4 cells, and activated human lymphocytes but not in HPB-ALL or Jurkat cells, or human hepatocytes. From these results, Nakayama *et al*. (14) concluded that R-125224 has a cell selectivity in inducing apoptosis. Under the same conditions as those employed in the present study, they also determined the K_d value in binding of ^{125}I-R-125224 to the activated human lymphocytes, which was 4.27 ± 0.05 nM. This value is very close to the K_d value in the human synovial cells obtained in the present study (1.32 ± 0.62 nM).

It was reported that the Fas antigen is expressed on the surface of synovial cells and lymphocytes (19). Interaction of Fas antigen and anti-Fas antibody led to apoptosis in various cells including inflammatory synovial tissues (20–24). Ogawa *et al*. (10) also showed that R-125224 treatment significantly reduced the number of activated human Fas$^+$T cells in SCID mice. Thus, the specific binding of ^{125}I-R-125224 *in vitro* and gradually increasing distribution of ^{125}I-R-125224 to transplanted synovial tissue in SCID mice *in vivo*, as observed in the present study, suggested that R-125224 would have a therapeutic effect on RA clinically.

The tissue distribution *in vivo* showed that ^{125}I-R-125224 was mainly present in the liver, spleen, adrenal grand and lymph node at 1 h post-dose (Fig. 4). We assume that the uptake of ^{125}I-R-125224 by these mouse tissues is not mediated by a binding to Fas antigen, since R-125224 showed almost no affinity to the mouse Fas antigen (Fig. 3). Lub-de Hooge *et al*. (25) reported that ^{111}In-DTPA-trastuzumab, the anti-HER2 receptor antibody, showed marked radioactivity uptake in the liver, spleen and kidney in human tumor-bearing mice. The distribution of ^{111}In-DTPA-trastuzumab is very similar to that of ^{125}I-R-125224, suggesting that the distribution profiles of humanized antibodies in mice are mediated by a similar mechanism.

The elimination of ^{125}I-R-125224 from transplanted synovial tissue was not parallel to the elimination from the plasma while that from other tissues was parallel to the elimination from the plasma. Poor blood flow might be a reason for a gradual increase of radioactivity in the transplanted synovial tissues, while the brown adipose tissue, which is located under the skin and is quite unlikely to have a rich blood flow, showed a decrease of the concentration in parallel to that from the plasma. Therefore, a poor blood flow could not be a reason for a long retention of the radioactivity in the synovial tissues. Based on this observation as well as on the facts that a high affinity of R-125224 to human synovial cells (K_d: 1.32 nM, Table I) and no affinity of R-125224 to mouse Fas (Fig. 3), we concluded that this antibody is distributed specifically to transplanted synovial tissues. As shown in Figs. 6 and 7, more than 60% of the radioactivity in transplanted synovial tissue occurred as the intact form of R-125224 over a period up to 168 h post-dose. This observation indicates that a long retention of the radioactivity almost as the intact R-125224 takes place by binding to Fas antigen and that R-125224 on the surface of synovial tissue is internalized very slowly otherwise undergoing degradation into smaller metabolites. In the liver, on the other hand, most of the radioactivity distributed was degraded and occurred as the metabolites with low molecular weights. Detailed mechanism for the disposition of antibodies has not been clarified yet, but internalization of antibodies into cells has been reported. Lub-de Hooge *et al*. (25) examined the internalization of ^{111}In-DTPA-trastuzumab using HER2-overexpressing human ovarian SK-OV-3 tumor cell line. They added ^{111}In-DTPA-trastuzumab to SK-OV-3 cells and incubated the mixture at 4°C for 1 h. After the incubation, the cells were assayed for the surface-bound, intracellular and supernatant radioactivities. The intracellular radioactivity was increased in a time-dependent manner as the surface-bound radioactivity decreased gradually. They concluded that ^{111}In-DTPA-trastuzumab was internalized through the HER2 receptor. Coffey *et al*. (26) reported that murinized anti-mouse CD11a monoclonal antibody, muM17, was cleared *in vivo* by binding to peripheral blood mononuclear cells, lymphocytes and splenocytes and by subsequent internalization and lysosomal degradation. Considering that characteristically R-125224 has no affinity to the mouse Fas antigen, extensive metabolism of ^{125}I-R-125224 in mouse liver strongly suggests that R-125224 binds to a cell surface receptor other than the Fas antigen, and is internalized through a receptor-mediated endocytosis. This suggests that the distribution profile of R-125224 in SCID mice would differ significantly from that in animal species expressing the Fas antigen's cross-reactivity to R-125224 since the Fas antigen is additionally involved in the internalization. A follow-up experiment using such animal species expressing the cross-reactive Fas antigen, a tissue distribution study using monkeys with collagen-induced arthritis is ongoing, the results of which will be described elsewhere.

CONCLUSION

The current study demonstrates that a humanized anti-human Fas monoclonal antibody, R-125224, has high affinity to human synovial cells and is distributed to the pharmacological target, synovial tissue, in SCID-HuRAg mice.

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