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Distribution of Immunoglobulin Fab Fragment Conjugated with HIV-1 REV Peptide following Intravenous Administration in Rats

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Abstract: HIV-1 REV peptide (positions 34−50) is well-known as a cell-permeating peptide. In this study, we investigated the distribution of Fab fragment of immunoglobulin conjugated with REV peptide (REV-Fab) following intravenous administration in rats, and compared with those of the native Fab fragment (nFab). Radioiodinated REV-Fab or nFab (^{125}I-REV-Fab or ^{125}I-nFab, respectively) was given in a single intravenous dose of 2 mg/kg (3 MBq/kg). Total radioactive and TCA-insoluble radioactive concentrations in blood, whole-body autoradiography (ARG), and urinary excretion rates were assayed following administration. Regarding blood and plasma, total radioactive and TCA-insoluble radioactive concentrations for ^{125}I-REV-Fab were remarkably lower than those for ^{125}I-nFab. In the whole-body ARG at 4 h after administration, ^{125}I-REV-Fab produced remarkably higher radioactivity in the adrenal gland, spleen, and liver, compared to ^{125}I-nFab. Regarding urinary excretion rates, approximately 70% of the radioactive dose was excreted in the form of a low-molecular-weight component by 24 h after administration for both samples. ^{125}I-REV-Fab may penetrate quickly from blood to adrenal gland, spleen, liver, and other tissues after intravenous administration to rats, and then did not stay in situ and was digested and excreted mostly via the renal route by 24 h. With these features, cell-permeating peptides are expected to help the development of new antibody pharmaceuticals.

Keywords: Whole-body autoradiography; HIV-1 derived REV peptide (positions 34−50); immunoglobulin Fab fragment; intravenous administration; cell-permeating peptide

Introduction

Immunoglobulin preparations contain many kinds of antibodies against viruses, bacteria, and the like and are widely used for the prevention and treatment of infectious diseases. Although these antibodies are effective in blood vessels and humoral fluids, they are unable to be fully effective, due to their inability to permeate the cell membrane of infected cells, against viruses, bacteria, and the like that have gone into latency in cells or tissues after infection. In recent years, many studies have been reported on the new finding that a series of arginine-rich peptides allow polymeric substances to penetrate into cells.[1−5] Against this background,

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we are studying such cell-permeating peptides with the aim of their application to the development of new drugs having the potential for penetration into cells and tissues conferred by binding immunoglobulin and the peptides.

Of the various cell-permeating peptides, the peptide derived from the TAT protein, an HIV-1 transcription factor, has been studied extensively and reported to show very quick penetration into cells and tissues. Studies of the pharmacokinetics and tissue distribution of this peptide are ongoing, and research programs aiming at the clinical application of drugs incorporating the peptide are being implemented.[6−10] Meantime, taking note of the identity of the TAT peptide as an arginine-rich sequence, we have shown that some new peptides are also capable of penetrating into cells.[11−15] The HIV-1-derived REV protein, in particular, like the TAT protein, is known to play a key role in nuclear export of the mRNAs,[16] and we showed that the peptide corresponding to positions 34−50, the site for binding to nucleic acid (RRE),[17] is highly capable of penetrating into cells.[11] In this study, we attempted to add a new function of intracellular penetration to immunoglobulin by binding it to the REV peptide.

There are only a very few studies of complexes of the REV peptide and high-molecular-weight proteins, with almost no reports available on complexes of the REV peptide and immunoglobulin or its functional fragment. Furthermore, although some studies are available on the penetration into cells in vitro, there are almost no reports on the distribution or tissue penetration. So, we conjugated the REV peptide to the Fab fragment of immunoglobulin and gave the resulting complex to rats by a single intravenous administration to examine its disposition. The complex of the REV peptide and the Fab fragment was prepared using a convenient method based on chemical modification,[11] rather than a gene recombination procedure. The Fab fragment was prepared from a polyclonal antibody preparation containing many different kinds of antibodies,[18] and was labeled with ^{125}I by the chloramine-T method.[19,20] Its distribution was analyzed by whole-body autoradiography, a method known to serve as an excellent tool for the accurate analysis of the kinetics of labeled drugs in animal bodies, and changes in its concentrations in blood and plasma over time and its urinary excretion were also determined.

Methods and Materials

Preparation of Immunoglobulin Fab Fragment. The immunoglobulin Fab fragment (nFab) used was prepared from a commercially available human polyclonal antibody preparation (Venoglobulin-IH, Mitsubishi Pharma, Japan) using the ImmunoPure Fab Preparation Kit (Pierce Chemical Company, USA).

Conjugation of REV Peptide to nFab. The REV peptide (TRQAR RNRRR RWRER QRGC) was chemically synthesized by the Peptide Institute Inc. (Osaka, Japan). Its purity was 95.1% as determined by HPLC. Conjugation of nFab with REV peptide was similarly carried out as reported previously.[11] An *N*-(6-maleimidocaproyloxy) succinimide

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ester (EMCS) solution, equivalent to 120 μg of EMCS, was added to 1 mL of 1 mg/mL nFab solution, and this mixed solution was gently stirred at room temperature for 2 h. The reaction liquor was treated with a PD-10 column (Amersham), equilibrated previously, to remove excess EMCS. Subsequently, the column effluent was concentrated by centrifugation using Microprep (Millipore, USA), and phosphate-buffered saline (PBS) was added to make a final volume of 1 mL. A REV peptide solution, equivalent to 600 μg of the peptide, was added to this solution, and the mixture was gently stirred at room temperature for 2 h. The excess portion of the REV peptide was removed using a PD-10 column, the column effluent was concentrated by centrifugation using Microprep (Millipore), PBS was added to make a final concentration of 1 mg/mL, and this solution was stored under refrigeration until use.

Iodine Labeling of REV-Fab or nFab. The REV-Fab or nFab fraction was labeled with ^{125}I by the chloramine-T method.[19,20] Twenty micrograms of protein was incubated with 37 MBq (1 mCi) of [^{125}I]Na (Amersham, France) and chloramine T (10 nmol) in Eppendorf tubes for 30−60 s at room temperature. The reaction was quenched by the addition of sodium meta-bisulfite (62 nmol). Free iodine was removed by chromatography on a PD-10 Sephadex G-25 column (Amersham, Les Ulis, France). Iodine labeling of REV-Fab or nFab was prepared by the addition of nonlabeled REV-Fab or nFab to 2 mg/mL. Specific activity ranged about 1.5 MBq/mg for REV-Fab or nFab.

Uptake into HeLa cells. HeLa cells were seeded on 24-well plate at a density of 2.5−5.0 $\times$ 10^4 cells per well. After a subconfluent state was reached, the culture medium was replaced with 500 μL of MEM medium, and the plate was allowed to stand in an incubator at 37 °C for 3 h. Subsequently, 5 μL of the test substance was added and reacted under the specified conditions, after which the plate was washed three times by PBS, a trypsin-EDTA solution was added, and the reaction was carried out at 37 °C for at least 15 min to completely detach the cells. The cells and cell lysate were recovered from each well to a polystyrene tube, and radioactivity was determined using a gamma counter (COBRA Quantum; PerkinElmer, Meriden, CT).

Intravenous Administration of REV-Fab or nFab. Male Sprague-Dawley rats were given free access to food and water. Each rat was weighed (288−307 g) and housed in a metabolic cage for collection of urine and feces at 24 h. Rats received a single bolus dose of 2 mg/kg (3 MBq/kg, 80 μCi/kg) of ^{125}I-REV-Fab or ^{125}I-nFab via the femoral vein. This dosage was selected to be identical with that employed for the clinically used antibody drugs.[21]

Determination of Blood and Plasma Concentrations. At 0.25, 0.5, 1, 3, 6, and 24 h after administration of the test substance, blood (approximately 0.4 mL) was drawn from the caudal vein using a glass capillary having its inner wall treated with 500 units/mL heparin sodium in physiological saline. A 0.1 mL portion of each blood sample was separated for determination of blood concentrations, and the remaining portion was quickly centrifuged (3000 rpm, 15 min, 4 °C) to separate plasma. These blood samples were stored under ice-cooling conditions until plasma separation. Prior to every time of blood drawing, the animals were examined for macroscopic signs. A 0.1 mL portion of blood or plasma was transferred to a polystyrene tube, and the radioactivity in the sample was determined using a gamma counter. Subsequently, 1 mL of physiological saline and 1 mL of 20% TCA were added to the assayed sample, and this was followed by centrifugation (3000 rpm, 15 min, 4 °C). The supernatant was removed, 1 mL of physiological saline was added to the sediment, followed by centrifugation in the same manner, the supernatant was removed, and the radioactivity in the TCA sediment fraction was determined.

Whole-Body ARG. Animals receiving the test substance were killed by ether anesthesia at 4 h after administration. After each animal was clipped, an approximately 3% carboxymethylcellulose sodium salt (CMC-Na) solution was used to fill in the oral cavity, nasal cavities, ear holes, and anus, and the animal was quickly frozen in a hexane−Dry Ice bath. The tail and extremities were then amputated, and the body was preserved in wrap at −20.4 to −17.0 °C for at least 24 h. An approximately 3% aqueous solution of CMC-Na was applied to the body surface of the frozen fixed rat. Subsequently, the body was embedded in an approximately 5% aqueous solution of CMC-Na and frozen in a hexane−Dry Ice bath to obtain a frozen block. Frozen sections 30 μm in thickness were prepared using a cryomicrotome. The sections obtained involved a median plane, a renal plane, and 2 planes of major tissues. Each section was freeze-dried at approximately −20 °C. The dry section was applied to base paper and covered with Mylar film. This section for ARG was brought into close contact with an imaging plate (IP) and exposed to radiation in a shield box for 12 h. After completion of the exposure, readings were taken using a bioimaging analyzer, and a whole-body ARGM was generated (gradation, 65 536; resolution, 50 μm; sensitivity, 4000; latitude, 5; IP, BAS-MS2040). Images obtained by whole-body ARG were analyzed on a relative basis using the Image Gauge software program (version 3.46, Fuji Photo Film, Japan).

Determination of Urinary Excretion. Animals receiving the test material were placed in metabolic cages, and all urine was pooled for a period of 0−24 h after administration (1 plot in total). These urine samples were collected under ice-cooling conditions. After urine sampling, the inside of the metabolic cage was washed with an appropriate amount of 50% methanol, and the washings were recovered. The volumes of urine and cage washings were quantified, and, as required, purified water was added for the purpose of

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dilution. A 0.2 mL portion of this urine sample and washings was transferred to a polystyrene tube, and the radioactivity in the sample was determined using a gamma counter. Subsequently, 1 mL of physiological saline and 1 mL of 20% TCA were added to the assayed sample, and this was followed by centrifugation (3000 rpm, 15 min, 4 °C). The supernatant was removed, 1 mL of physiological saline was added to the sediment, followed by centrifugation in the same manner, the supernatant was removed, and the radioactivity in the TCA sediment fraction was determined.

Excretion rates were determined as the percent ratio of radioactivity in urine and washings to the dosed radioactivity. After background was subtracted from the measured values of radioactivity in urine and washings, the ratio to the dosed radioactivity (actual measured value) was calculated. The excretion rates of the radioactivity in the washings and the radioactivity in urine were separately calculated, and the two values were summed to obtain the overall urinary excretion rate.

Results

Penetration of Test Samples into HeLa Cells. ^{125}I-nFab or ^{125}I-REV-Fab was added to the culture medium for subconfluent HeLa cells, and their uptake into the cells was examined. Figure 1A shows the dose dependent curves for ^{125}I-nFab and ^{125}I-REV-Fab to incorporate in the cells. The cell-associated protein level of ^{125}I-REV-Fab was increased with dose dependence from 1.25 nM to 400 nM, but that of ^{125}I-nFab was very low to 400 nM. As shown in Figure 1B for time-related changes in uptake, ^{125}I-REV-Fab was incorporated in the cells in a very short time, but the incorporation level of ^{125}I-nFab was very low for 60 min. In this experiment, the cells were treated with trypsin prior to the radioactivity measurements. This trypsin treatment effectively removes the surface-adsorbed proteins,[16] and therefore, the majority of the radioactivity can be attributed to the internalized Fabs. The internalization of fluorescently labeled REV-Fab was also confirmed by confocal microscopic observation (data not shown). The effectiveness of the REV segment as a delivery vector was thus verified.

Radioactive Concentrations in Blood and Plasma. Figures 2 and 3 shows total radioactive concentrations in blood and plasma and radioactive concentrations in the TCA-insoluble fraction following intravenous administration of ^{125}I-nFab or ^{125}I-REV-Fab.

Following administration of ^{125}I-nFab, the total radioactive concentration in blood was 9149 ± 324 ng equiv/mL at 0.25 h after administration, the first measuring time point, after which the concentration decreased in a biphasic pattern and became 274 ± 29 ng equiv/mL at 24 h after administration. The radioactive concentration in plasma was 16 839 ± 1643 ng equiv/mL of ^{125}I-nFab at 0.25 h, after which the concentration decreased in a biphasic pattern and became 377 ± 37 ng equiv/mL at 24 h. The ratio of TCA-insoluble radioactive concentration to total radioactivity for ^{125}I-nFab was approximately 92.0% and 93.2% in blood and plasma at 0.25 h, respectively, after which it decreased until 6 h and became approximately 36.5% and 22.6%, respectively, and then, it recovered to 69.3% and 60.3% at 24 h, respectively (Table 1).

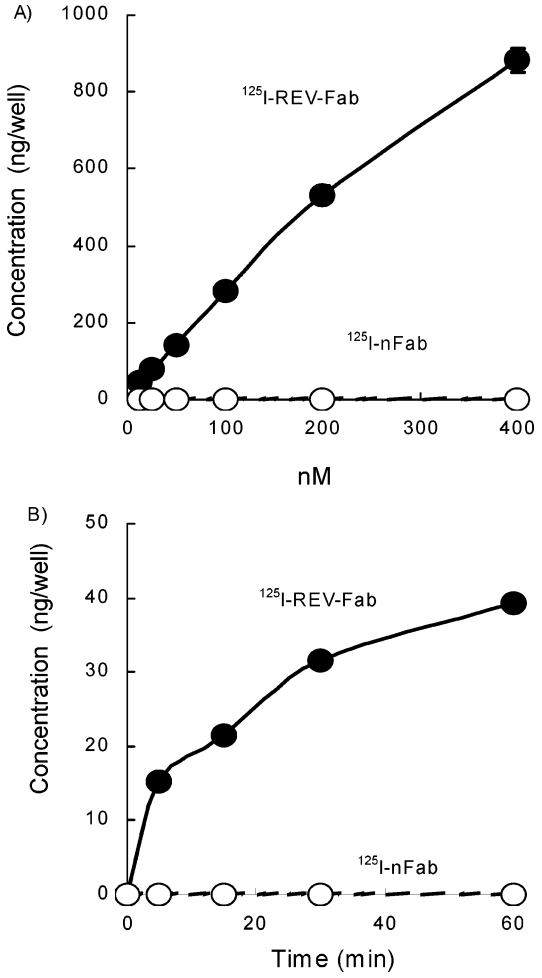

Figure 1. Uptake of ^{125}I-REV-Fab or ^{125}I-nFab into HeLa cells. (A) Concentration−response curve for ^{125}I-REV-Fab (●) or ^{125}I-nFab (○). HeLa cells were incubated for 30 min in the medium containing test sample with concentrations from 1.25 to 400 nM (corresponding to 0.04 and 12 μg of protein/well, respectively). (B) Time course of response to ^{125}I-REV-Fab (●) or ^{125}I-nFab (○). HeLa cells were treated with antibodies at a concentration of 20 nM (0.6 μg of protein/well). At different time points the cells were washed and trypsinized. The cell-associated ^{125}I-proteins (ordinate) were estimated from radioacitivity of trypsinized cell and cell lysate, reported per well of a 24-well plate, and presented as mean ± SD of four wells.

Following administration of ^{125}I-REV-Fab, the total radioactive concentration in blood was 2619 ± 767 ng equiv/mL at 0.25 h, a level approximately $^{1}/_{3.5}$ of the concentration observed with ^{125}I-nFab, after which the concentration decreased in a biphasic pattern and became 356 ± 92 ng equiv/mL at 24 h after administration. The total radioactive concentration in plasma decreased to 3949 ± 772 ng equiv/mL, a level approximately $^{1}/_{4.3}$ of those with ^{125}I-nFab, and this was followed by a biphasic reduction. The ratio of TCA-insoluble radioactive concentration to total radioactivity for ^{125}I-REV-Fab in blood was approximately 83.9% and 80.7%

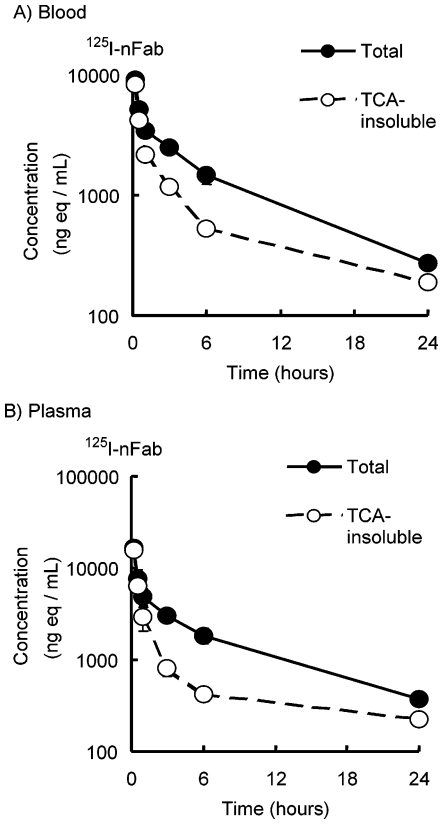

Figure 2. Concentrations of radioactivity in blood and plasma after intravenous administration of ^{125}I-nFab to male rats. Total radioactivity (●) and the radioactivity of TCA-insoluble pellets (○) were measured by a gamma counter. Data are the mean ± SD of the results from 3 animals. Concentration of radioactivity is shown as the corresponding amount of ^{125}I-labeled proteins yielding equivalent radioactivity (ng equiv/mL).

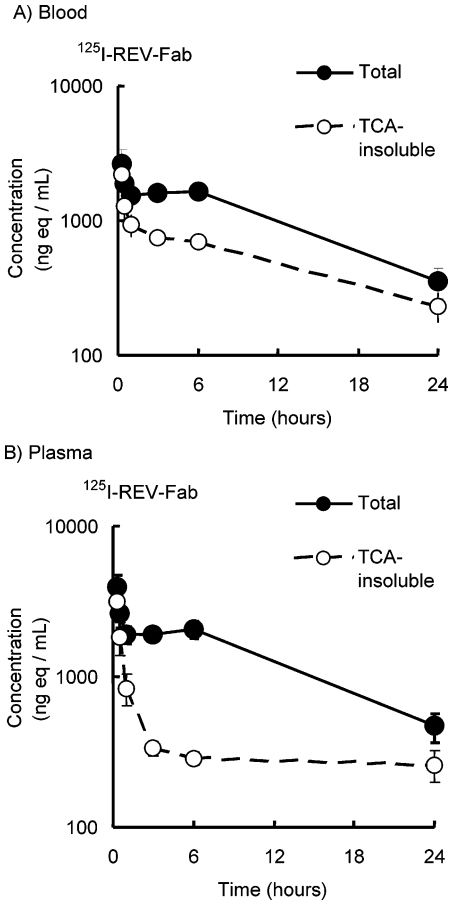

Figure 3. Concentrations of radioactivity in blood and plasma after intravenous administration of ^{125}I-REV-Fab to rats. Total radioactivity (●) and the radioactivity of TCA-insoluble pellets (○) were measured by a gamma counter. Data are the mean ± SD of the results from 3 animals. Concentration of radioactivity is shown as the corresponding amount of ^{125}I-labeled proteins yielding equivalent radioactivity (ng equiv/mL).

in blood and plasma, respectively, at 0.25 h, after which it decreased until 6 h and became approximately 42.9% and 13.9%, respectively, and then it recovered to 65.6% and 55.4% respectively (Table 2).

Whole-Body Autoradiography. Radioactivity distributions at 4 h after intravenous administration of ^{125}I-nFab or ^{125}I-REV-Fab are shown in Figures 4 and 5, respectively. First, for ^{125}I-nFab, particularly high radioactivity was observed in the thyroid, renal cortex, intracystic urea, and gastric contents, and relatively high radioactive concentrations were observed in the skin, whereas almost no radioactivity was detected in the brain, spinal cord, or eyeballs. For ^{125}I-REV-Fab, high radioactive concentrations were detected in the thyroid, renal cortex, gastric contents, intracystic urea, and skin, whereas almost no radioactivity was detected in the brain, spinal cord, or eyeballs, as with ^{125}I-nFab. However, for ^{125}I-REV-Fab, high radioactive concentrations were also observed in the spleen, liver, and adrenal gland.

Urinary Excretion. Table 3 shows the urinary radioactive excretion rates following intravenous administration of ^{125}I-nFab or ^{125}I-REV-Fab. The urinary excretion rates for ^{125}I-nFab and ^{125}I-REV-Fab were 71.2 ± 14.8% and 69.5 ± 4.5%, respectively, by 24 h after administration. The ratio of TCA sedimentable fraction to total urinary radioactivity was 0.38% and 0.04%, respectively. Therefore, the majority of radioactivity would be derived from low-molecular-weight components containing free or peptide-bound ^{125}I, because the TCA-insoluble radioactivity corresponds to the intact

Table 1. TCA-Insoluble Radioactivity Ratio after Intravenous Administration of ^{125}I-nFab to Male Rats

time after administration[a] (h)	TCA-insoluble radioactivity ratio (%)[b]	
	in blood	in plasma
0.25	92.0 ± 4.8	93.2 ± 0.7
0.5	81.8 ± 7.6	83.7 ± 2.4
1.0	63.8 ± 1.6	58.9 ± 4.4
3.0	46.9 ± 1.4	26.5 ± 3.4
6.0	36.5 ± 4.6	22.6 ± 0.7
24.0	69.3 ± 4.2	60.3 ± 6.0

[a] Time after intravenous administration of ^{125}I-nFab. [b] The ratio of TCA-insoluble radioactivity per total radioactivity in blood or plasma. Data are the mean ± SD of the results from 3 animals.

Table 2. TCA-Insoluble Radioactivity Ratio after Intravenous Administration of ^{125}I-REV-Fab to Male Rats

time after administration[a] (h)	TCA-insoluble radioactivity ratio (%)[b]	
	in blood	in plasma
0.25	83.9 ± 1.7	80.7 ± 1.7
0.5	63.3 ± 1.3	69.2 ± 4.9
1.0	60.6 ± 3.2	44.0 ± 5.2
3.0	46.6 ± 1.6	17.6 ± 0.5
6.0	42.6 ± 1.1	13.9 ± 1.3
24.0	65.6 ± 0.9	55.4 ± 5.3

[a] Time after intravenous administration of ^{125}I-REV-Fab. [b] The ratio of TCA-insoluble radioactivity per total radioactivity in blood or plasma. Data are the mean ± SD of the results from 3 animals.

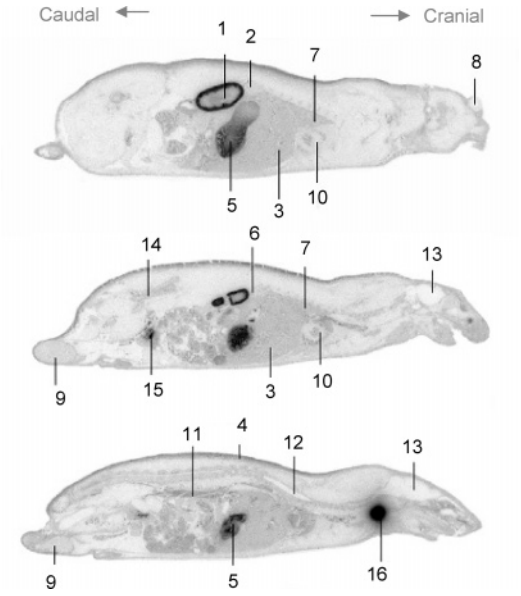

Figure 4. Whole-body autoradiograms at 4 h after intravenous administration of ^{125}I-nFab to male rat. Each number indicates responsible organs. 1: Kidney. 2: Spleen. 3: Liver. 4: Skin. 5: Gastric contents. 6: Adrenal gland. 7: Lung. 8: Eyeball. 9: Testis. 10: Heart. 11: Blood. 12: Spinal cord. 13: Brain. 14: Bone marrow. 15: Urinary bladder. 16: Thyroid gland.

REV-Fab as well as partially digested proteins having relatively high molecular weights.

Discussion

A recent study has revealed that high-molecular-weight substances can be incorporated in cells through the cell membrane when bound with certain basic peptides, though they are otherwise unable to penetrate into cells.[22−24] On the TAT peptide, in particular, many reports are available, including a great deal of research into its kinetics in animal

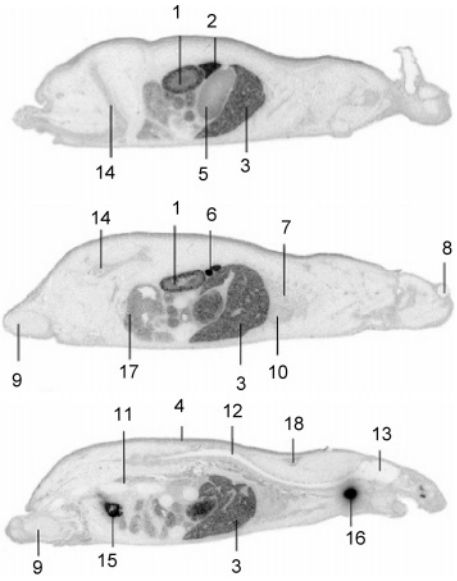

Figure 5. Whole-body autoradiograms at 4 h after intravenous administration of ^{125}I-REV-Fab to male rat. Each number indicates responsible organs. 1: Kidney. 2: Spleen. 3: Liver. 4: Skin. 5: Gastric contents. 6: Adrenal gland. 7: Lung. 8: Eyeball. 9: Testis. 10: Heart. 11: Blood. 12: Spinal cord. 13: Brain. 14: Bone marrow. 15: Urinary bladder. 16: Thyroid gland. 17: Intestinal contents. 18: Brown fat.

Table 3. Urinary Excretion of Radioactivity in 24 h after Intravenous Administration of ^{125}I-REV-Fab or ^{125}I-nFab to Male Rats

samples	urinary excretion	
	% of dose[a]	% of TCA-insoluble[b]
^{125}I-REV-Fab	69.5 ± 4.5	0.04 ± 0.00
^{125}I-nFAB	71.2 ± 14.8	0.38 ± 0.15

[a] Total urinary excretion of radioactivity per total radioactivity of dose. [b] The ratio of TCA-insoluble radioactivity per total urinary radioactivity. Data are the mean ± SD of the results from 3 animals.

bodies and in blood, as well as intracellular uptake,[25−27] and studies are ongoing to apply it to the treatment of various diseases.[28−31] Additionally, since the TAT peptide has an

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arginine-rich sequence, other arginine-rich peptides of protein origin have also been studied extensively; peptides from the HIV-1 REV protein or the FHV coat,[32] like the TAT peptide, have been shown to be capable of allowing high-molecular-weight substances to penetrate into cells.

The REV protein is well-known as a nuclear exporter of the mRNAs, and its activity is of paramount importance to the proliferation of the virus; accordingly, many reports have been presented on the suppression of its activity. On the other hand, we found that the peptide corresponding to position 34−50, which is important to the transcription activity of the REV protein, not only serves as a nucleic acid binding site but also is capable of permeating the cell membrane. We are now attempting to add a new function of intracellular penetration to antibodies by conjugating them with the REV peptide.

In the present study, we first examined the uptake of ^{125}I-labeled REV-Fab complex in HeLa cells, and verified the efficient and quick incorporation of the ^{125}I-labeled complex into the cells with the help of the REV peptide. Subsequently, the disposition and blood kinetics following intravenous administration in rats were investigated using this RI-labeled complex. The blood and plasma concentrations of ^{125}I-REV-Fab decreased very rapidly in the initial phase following intravenous administration; conversely, in the terminal phase, ^{125}I-REV-Fab decreased more slowly than ^{125}I-nFab. Regarding whole-body ARG, the time point for comparison was set to be 4 h after administration, when the initial rapid change had been complete and a transition to gradual decline occurred. We demonstrated that ^{125}I-REV-Fab produced remarkably greater accumulation of radioactivity in the adrenal gland, spleen, and liver compared with ^{125}I-nFab. The rapid initial decreases in blood and plasma concentrations following administration of ^{125}I-REV-Fab can be explained by the penetration of ^{125}I-REV-Fab to these organs, resulting in significantly lower concentrations than those of ^{125}I-nFab. The slow elimination from plasma in the terminal phase is also attributable to the gradual liberation of tissue-incorporated ^{125}I-REV-Fab complex from the tissue to blood. Since the TCA-insoluble fraction content was high at approximately 90% in the initial phase after administration but decreased to approximately 60% in the terminal phase, and also since the TCA sediment fraction content in urine decreased to less than 1%, it was postulated that ^{125}I-REV-Fab underwent decomposition to low-molecular-weight components in tissue and then excreted in urine. These results suggested that the incorporated labeled complex did not stay in situ for a long time, with approximately 70% of the dose excreted via the renal route in the form of low-molecular-weight components by 24 h after administration. TCA-insoluble radioactivity ratios were decreased over a 6 h period after iv injection, but then increased at 24 h. The total radioactivity decreased consistently between 6 and 24 h after iv injection, whereas the decrease in TCA-insoluble radioactivity was not so significant. This eventually yielded the apparent increase in the TCA-insoluble radioactivity ratios at 24 h after injection.

Several reports of studies using the TAT peptide show high pulmonary uptake, as well as accumulation in the spleen and liver,[6−8] whereas the results of the present study for ^{125}I-REV-Fab showed almost no uptake in pulmonary tissue. This difference is partially attributable to the fact that the reports on the use of the conventional TAT peptide handled the peptide as is or its complex with a substance of relatively low molecular weight. Additionally, it has been reported that a complex of TAT and β galactosidase was incorporated in the brain as of 8 h after administration when given by intraperitoneal administration.[6] In the case of ^{125}I-REV-Fab, however, almost no radioactivity was observed in the brain, spine, or eyeballs, though the determination was made at 4 h after administration. It remains unclear whether these differences are due to differences in the kind of protein modified, or to differences in the kind of cell-membrane-permeating peptide used.

The above results demonstrate that ^{125}I-REV-Fab penetrates into organs in a very short time following intravenous administration in vivo, as well as its intracellular penetration in vitro. Furthermore, its disposition was found to occur in accumulative pattern in particular organs, such as the spleen, liver, and adrenal gland, rather than in a uniform distribution to all organs. It was also shown that the incorporated labeled complex did not stay in situ for a long time, with approximately 70% of the dose excreted via the renal route in the form of low-molecular-weight components by 24 h after administration. With these features, cell-permeating peptides are expected to help the development of new antibody pharmaceuticals.

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