1). Average pixel counts derived from regions of interest on the reconstructed emission images were referred to the well counter radioactivity counting rate, to determine the cross-calibration factor between the SPECT images and well counter system. This cross-calibration factor was subsequently used for the blood sample counts of the clinical studies. Uniformity of the reconstructed emission images was evaluated.

Clinical Studies

All clinical SPECT studies followed the DTARG protocol, with dual administration of iodoamphetamine (*23*), depicted in Figure 1. Briefly, 2 dynamic scans were acquired in quick succession, with a 2-min interval between the scans. The first scan covered the initial 0- to 28-min period, and the second was acquired from 30 to 58 min. At 4 min per frame, 7 frames covered each of the 2 dynamic scan periods. ^{123}I-iodoamphetamine (111 MBq at institutions 2–12 or 167 MBq at institution 1) was infused twice over 1 min into the antecubital vein at 0 and 30 min. Acetazolamide (17 mg/kg, 1,000 mg maximum) was administered intravenously at 20 min after the first iodoamphetamine injection, corresponding to 10 min before the second iodoamphetamine injection. Projection data were summed for the acquisition duration of the first and second scans and reconstructed as described in the "QSPECT Reconstruction" section. In contrast to the study of Kim et al. (*23*), which used full arterial blood sampling, the individual arterial input functions were derived from a population-based standardized input function scaled with the whole-blood counts from a single arterial blood sample taken at approximately 10 min (*1,25–28*). This sample was also used for arterial blood gas analysis.

In the reproducibility arm, an additional, non-DTARG CBF study was performed on a separate day. Instead of DTARG, the previously reported ^{123}I-IMP autoradiographic (IMPARG) method (*1,19,25*) was performed within a month of the DTARG study. The IMPARG method is essentially equivalent to the present DTARG method, except that the IMPARG method uses a single iodoamphetamine administration to assess CBF either at rest or after acetazolamide challenge. The same image reconstruction process as for the DTARG protocol was used. In 12 studies, the DTARG protocol was used instead of IMPARG—namely, the DTARG study was performed twice to assess the CBF reproducibility at rest and after acetazolamide.

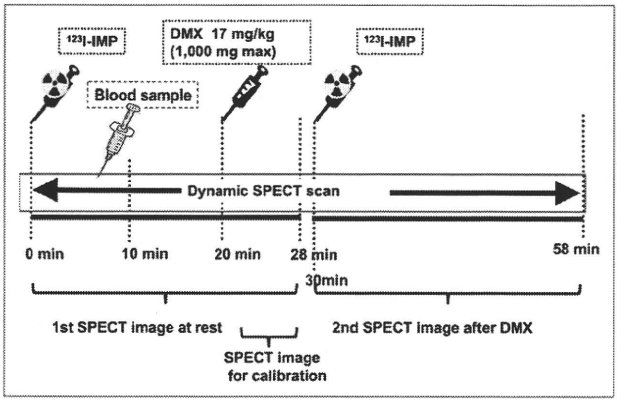

FIGURE 1. Scanning protocol flow for DTARG procedure. ^{123}I-iodoamphetamine was injected at 0 min, and 28-min resting dynamic SPECT scan was commenced. Blood sample for calibration of population input function was drawn at 10 min. Acetazolamide (DMX-diamox) was administered at 20 min. CBF values are scaled by last frame (time, 24–28 min). Second dynamic SPECT scan followed second injection of ^{123}I-iodoamphetamine at 30 min. IMP = iodoamphetamine.

In the versus-PET protocol, the PET study was performed within 2 d of the DTARG SPECT study. PET scans used intravenous ^{15}O-water both at rest and after the acetazolamide challenge. CBF images were calculated by the ^{15}O-water autoradiography technique (*29*), with careful corrections for delay and dispersion (*30–32*). Patients were stable between the SPECT and PET studies.

In the rest–rest protocol, the DTARG scan was obtained without the pharmacologic challenge during the study to evaluate the consistency of CBF values estimated from the 2 scans.

QSPECT Reconstruction

The program package for QSPECT uses a wrapper written in JAVA to run several programs written in C for Microsoft Windows systems. The package includes programs for reconstructing SPECT images, calculating functional images, coregistering images, and reslicing and printing summary logs.

The QSPECT package reconstructs images from the original projection data from commercial SPECT equipment, based on previous work by Iida and his colleagues (*19–21,23,33,34*). Reconstructed SPECT images are calibrated in Bq/mL, which provides independence from scanning parameters such as the acquisition time, number of views, matrix size, and zoom factor. Uniformity and center-of-rotation corrections and fanbeam–to–parallel beam conversion (for fanbeam collimators) were performed using the clinical routine software before reconstruction by this package.

An overall flow diagram of the correction and reconstruction process is shown in Supplemental Figure 1. The OSEM reconstruction technique includes attenuation correction (*17*). A threshold-based edge-detection algorithm generated the attenuation μ-map, assuming a uniform attenuation coefficient of 0.166 cm^{-1} for ^{99m}Tc (0.160 cm^{-1} for ^{123}I) as an average over the brain and skull (*19*). The threshold was optimized via the user interface to correctly define the brain outline. The attenuation μ-map was generated from the summed 0- to 28-min rest frame and was coregistered to the other images (*35*) reconstructed with filtered backprojection without attenuation or scatter correction. The attenuation μ-maps were forward projected to provide the transmission projection data for TDCS. The emission projections were scatter-corrected by the TDCS method, as originally proposed by Meikle et al. (*18*), and further optimized for realistic ^{99m}Tc, ^{201}Tl, and ^{123}I data in the brain and thorax regions (*20,21,23,33,34*). An offset compensated for the septal penetration of high-energy photons for ^{123}I studies, which adds fairly uniform background counts, or direct current (DC) components, to the projections.

Scatter- and attenuation-corrected images were reconstructed with OSEM (5 iterations, 5 subsets using geometric-mean projections, postreconstruction gaussian filter of 7 mm in full width at half maximum) and then realigned to the image set obtained from the first scan. The acquisition parameters and BCF were used to convert the reconstructed raw counts to Bq/mL.

The global CBF over the entire gray matter was estimated from the SPECT frame covering 24–28 min, because this timing minimizes the individual shape variations in individual input function. The look-up table generated for estimating CBF images from the complete dynamic study (0–28 min) was then

scaled to provide global cortical gray matter CBF values consistent with the 24- to 28-min frame estimates. A careful detection algorithm was used to reliably exclude extracranial accumulation of ^{123}I-iodoamphetamine (e.g., in the parotid region), which could adversely affect this scaling procedure. The regional CBF was then estimated at each pixel by means of the table look-up procedure (*25,28*). The background image at the time of the second ^{123}I-iodoamphetamine injection was estimated from the first-phase CBF images, according to the compartment model assumed in this study (*23*). An additional table look-up procedure was applied to the second dynamic dataset (30–58 min) for calculating the vasodilated (acetazolamide challenge) CBF images as described previously (*23*). The data were successfully reconstructed, and CBF was estimated at each institution. To facilitate and provide consistent analysis, the data presented are from the reanalysis conducted at the core lab (National Cerebral and Cardiovascular Center).

Data Analysis

The uniform phantom SPECT activity estimates were compared with the known activity in the phantom. Images for the baseline study were displayed with subsequent images using an absolute flow value scale to visually ascertain regional and global differences in flow. Regions of interest were placed on the middle cerebral artery territories of both hemispheres, and the average flow values between the different methods were compared and plotted. Bland–Altman plots and the SD of the differences evaluated the consistency of CBF values obtained from the reproducibility and versus-PET protocols.

All data were presented as mean ± SD. Pearson correlation analysis and linear regression analysis were used to evaluate relationships between the 2 CBF values. A P value less than 0.05 was considered statistically significant.

RESULTS

Phantom Studies

In the 16-cm scattering cylinder line source experiment, the scatter-uncorrected images show background counts extending beyond the phantom, from septal penetration of the high-energy photons. The scatter correction is largely effective in correcting for scatter and septal penetration counts. As shown in Supplemental Figure 2, the Toshiba-ECAM low- to medium-energy general-purpose (LMEGP) collimator, designed for reduced ^{123}I septal penetration, compared with the standard low-energy high-resolution collimator (GE Healthcare), demonstrates reduced scatter and septal penetration counts. The lower septal penetration of the Toshiba-ECAM LMEGP collimators is also supported by a lowered scatter correction offset value (DC = 0.05, compared with DC = 0.20 for the GE low-energy high-resolution collimator). The reduced scatter and septal penetration result in more complete removal of scatter for the LMEGP collimator.

Figure 2 displays reconstructed slices of the uniform phantom for all 12 institutions, scaled to the same maximum activity concentration. The estimated activity concentrations from these studies, compared with the known activity concentration, represented an accuracy of 87.5% ± 5.1% (Supplemental Table 1). The well counter–to–SPECT cross-calibration factor, which represents the sensitivity of the well counter system for ^{123}I, was 0.5–1.0 for NaI systems and 0.1–0.2 for plastic scintillation detector systems. The BCF values were consistent for the same SPECT camera–collimator configurations.

Clinical Studies

Figure 3A shows typical CBF images obtained at 4 institutions with 4 different γ-camera vendors, performed as part of the reproducibility arm of the study. Each case shows different CBF distributions both at rest and after acetazolamide challenge. The acetazolamide images obtained using the DTARG method agree well with the images subsequently obtained with the IMPARG method after acetazolamide infusion.

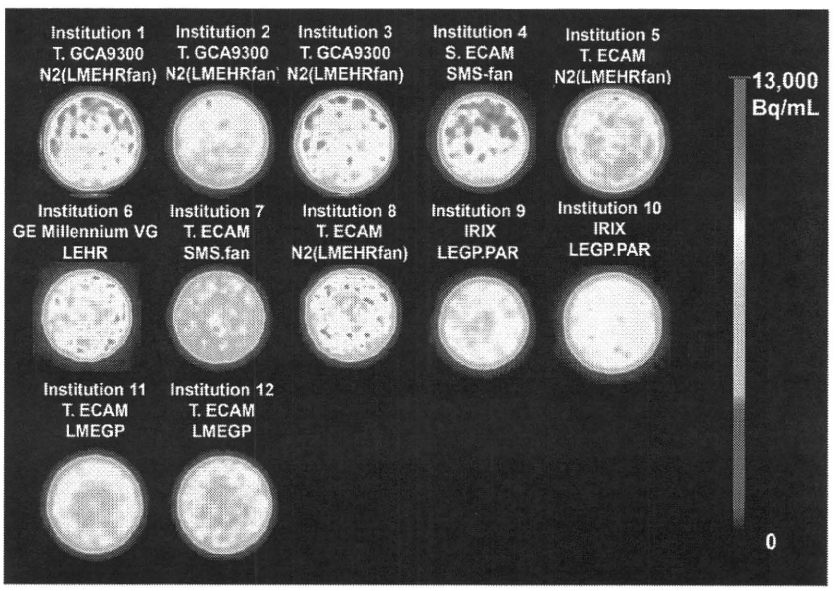

FIGURE 2. Reconstructed slices through uniform phantom from the participating 12 institutions. Experiment was designed to have same phantom activity concentration for each center's study. Nonuniformities and also differences in absolute activity concentration estimates can be observed, highlighting need for rigorous calibration, flood correction, and quality control. Legend above each image gives institution number (given in Supplemental Table 1), γ-camera model, and collimator used.

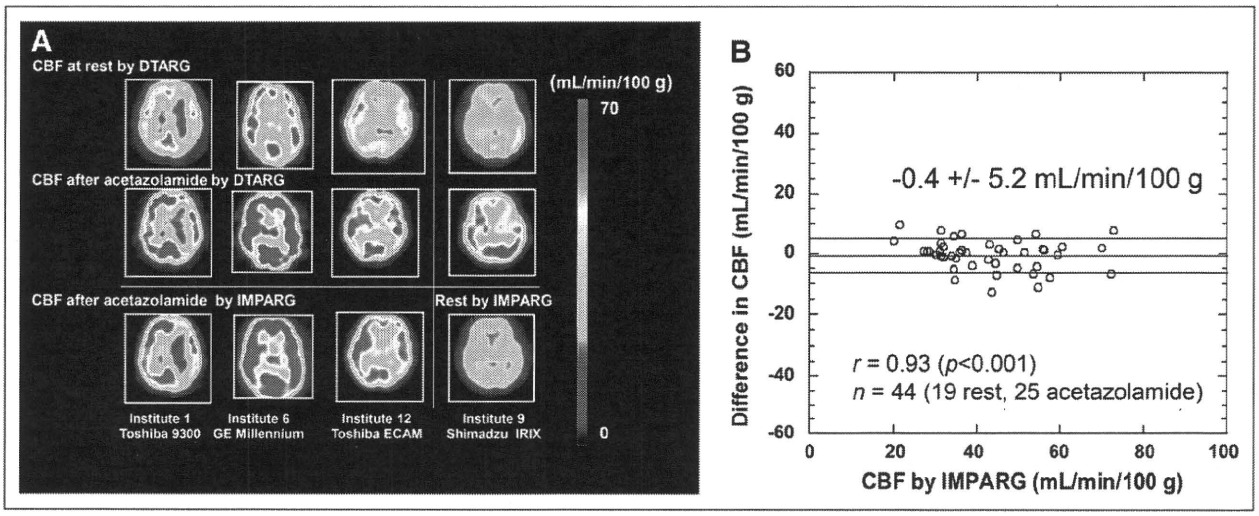

FIGURE 3. (A) Images from reproducibility study. CBF images obtained at rest and after acetazolamide with DTARG method. Repeated scan (third row) within 1 mo using IMPARG method and acetazolamide stress (columns 1–3) and at rest (last column). Images demonstrate that CVR can be estimated with this technique and demonstrate good reproducibility of measuring both at rest and after acetazolamide challenge CBF. (B) Bland–Altman plot showing difference vs. IMPARG CBF values estimated from DTARG method and repeated IMPARG studies to assess reproducibility. Little systematic bias is detected (mean difference, −0.4 mL/100 g/min), and SD of differences is moderate (5.2 mL/100 g/min). Correlation coefficient of $r = 0.93$ ($P < 0.001$) was found.

CBF images of a subject with left middle cerebral artery occlusion are shown in Supplemental Figure 3 for slices covering the whole brain. The images demonstrate reduced CBF after acetazolamide challenge in the left middle cerebral artery territory. The good reproducibility is confirmed by the Bland–Altman plot comparison of DTARG CBF values, with the CBF values obtained at a different imaging session with IMPARG or DTARG (Fig. 3B). The SD of the differences is 5.2 mL/100 g/min, with low bias supported by the mean difference of 0.4 mL/100 g/min. Regression analysis between DTARG and IMPARG values yielded a significant correlation ($P < 0.001$), with a correlation coefficient of $r = 0.93$.

Figure 4A shows MR and CBF images at rest and after acetazolamine obtained with DTARG SPECT and ^{15}O-water PET in a 73-y-old male patient (63 kg) with right internal carotid artery occlusion and left internal carotid stenosis. The MR images do not show any evidence of cerebral infarction in either hemisphere. Rest CBF was reduced bilaterally in the frontal-to-parietal regions, and acetazolamide increased CBF in left parietal regions but not in the right parietal area. DTARG CBF indicated the loss of vasoreactivity in the right internal carotid artery stenotic area. These findings were consistent with those from the PET evaluation. An additional example is shown in Supplemental Figure 4 for a 74-y-old female patient (48 kg) with left internal carotid artery stenosis, for whom MR images did not show cerebral infarction. DTARG CBF demonstrated preserved CBF in both hemispheres but reduced CBF reactivity in the left middle cerebral artery territory. The findings were again consistent with those from PET. Figure 4B compares the flow values obtained at rest and after acetazolamide with DTARG with the corresponding values obtained by ^{15}O-water PET. The SD of the differences is 5.1 mL/100 g/min, with the significant underestimation by ^{15}O-water PET, compared with PET by the DTARG method, highlighted by a mean difference of −6.1 mL/100 g/min. The Pearson analysis showed a significant correlation ($P < 0.001$), with a correlation coefficient of $r = 0.88$.

The results from the rest–rest protocol are summarized in Figure 5. The differences between the measurements performed with the 2 injections were small, with good agreement between the 2 flow values. The mean ± SD of the differences was 0.6 ± 2.9 mL/100 g/min.

DISCUSSION

The QSPECT package provided quantitative images consistent between the participating centers, using dual- or triple-detector SPECT scanners and collimators routinely used for nonquantitative brain studies. All centers successfully acquired the dynamic SPECT images, and the data from the variety of cameras encountered were successfully processed by the software package. Rest CBF and CVR could be readily obtained by the participating institutions in a single, clinically practical, 1-h scanning session. Good reproducibility of CBF estimates was observed in 31 pairs of studies at 8 institutions (Fig. 3), and the CBF estimated with the ^{123}I-iodoamphetamine SPECT agreed well with ^{15}O-water PET CBF at 1 institution (Fig. 4). The CBF values after the second injection of the DTARG were consistent with the values obtained after the

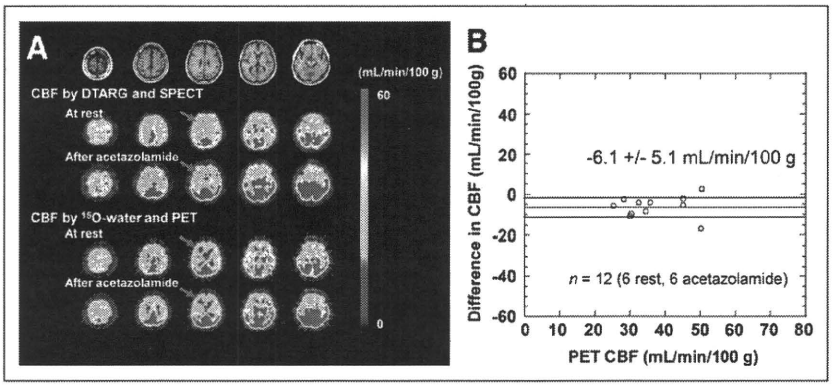

FIGURE 4. (A) MR and CBF images at rest and after acetazolamide stress assessed with corresponding measurements with ^{15}O-water PET (vs. PET evaluation) in patient with right internal carotid artery occlusion and left internal carotid stenosis. Gaussian filter was not applied to SPECT CBF in this display. (B) Bland–Altman plot. Moderate underestimation of CBF determined by DTARG method, compared with PET, is observed (mean difference, −6.1 mL/100 g/min). Correlation coefficient of $r = 0.88$ ($P < 0.001$) was found.

first injection when no vasodilating stress was given in 9 studies at 2 institutions (Fig. 5).

Quantitative CBF and CVR in response to acetazolamide challenge can be of significant prognostic value for patients considered for revascularization of cerebral arteries (*5–7*). The previously validated IMPARG method requires 2 independent scans on different days to assess the CVR (*5–7*), limiting it for routine clinical studies. The DTARG protocol to quantitatively assess CBF both at rest and after acetazolamide from a single dynamic SPECT session with the dual administration of ^{123}I-iodoamphetamine (*23*) facilitates clinical use. Errors caused by ambiguity in the absolute scaling, and possible changes in physiologic status of the subjects between scans, can be reduced substantially with the DTARG protocol. The quantitative reconstruction program enabled the compartment model–based kinetic analysis to compensate for the residual radioactivity concentration during the second session of the dynamic scan.

Major error sources in SPECT, namely attenuation and scatter, are only object-dependent (*14*) and not γ-camera– or collimator-dependent, and thus SPECT images obtained by this quantitative reconstruction package should be consistent across systems. Septal penetration of high-energy photons for ^{123}I is, however, collimator-dependent (*24*) but could be compensated as part of the TDCS scatter correction algorithm (*11*), as demonstrated in Supplemental Figure 2. The radioactivity concentration of the uniform cylinder phantom estimated in units of Bq/mL was consistent and showed variation within ±5.1% (Fig. 2; Supplemental Table 1), though a systematic underestimation by 12.5%, which is attributed to the BCFs being derived from a line source in air, reconstructed without scatter, attenuation, and septal penetration corrections. However, this underestimation does not affect the CBF estimation, because it relies on the direct cross-calibration between the γ-counter used to count the blood sample and the SPECT measurements.

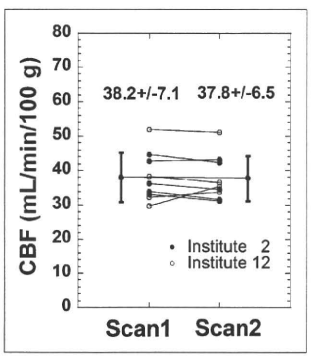

FIGURE 5. Results from rest–rest evaluation carried out at 2 institutions (2 and 12). In this study, DTARG method was performed as per normal protocol but without pharmacologic stress. CBFs estimated with first injection (left on graph) are in good agreement with those estimated after second injection (right on graph).

This phantom study also highlighted the importance of proper calibration and quality control of the γ-camera to avoid artifacts and bias in the reconstructed images. These corrections were applied, as for other clinical studies, by the vendors' software rather than as part of the QSPECT system, because these corrections are typically performed online and on-the-fly, with only the corrected data being stored. The nonuniformities seen on some phantom images should improve with more rigorous quality-control procedures.

The previously validated population-based input function requiring only a single arterial blood sample for scaling (*1,25–28*) has been incorporated in the software package. Blood from this single arterial sample is also used to measure arterial blood gases, which are relevant and of interest clinically in these patients. The timing of the single blood sample (~10 min after iodoamphetamine injection) was optimized previously (*1,25–28*) to minimize the errors associated with individual differences in shape of the arterial input function. In addition, absolute global CBF was estimated from SPECT images taken at an optimized mid scan time of approximately 30 min (24–28 min), rather than from the initial part of the study, to maximize the accuracy of using the population-based input function (*1,25–28*).

Partial-volume correction has not been implemented as part of this processing protocol. Partial-volume effects can potentially lead to underestimation of flow values in gray matter regions because of the limited resolution of SPECT. The small underestimation of 6.1 mL/100 g/min by the DTARG method, compared with ^{15}O-water PET (Fig. 4B), is attributed to the partial-volume effects due to differences in resolution between PET and SPECT. The underestimation can also lead to variations in CBF values obtained with different-

resolution collimators. However, consistent postreconstruction filtering, as applied in this study, can reduce this effect.

Only the reproducibility within an institution was assessed. Hence, the reproducibility of measurements between institutions cannot be gleaned from these data, particularly because patients with vascular disease were studied. Thus, unlike estimates from healthy volunteers, flow values and vascular reactivity are expected to vary from patient to patient, and flow values determined at one institution with one group of patients are therefore not directly comparable with flow values from another group of patients in another institution. A realistic brain phantom, such as recently developed by our group, simulating head contour with bone attenuation, could be used to assess the consistency of brain images between institutions.

CONCLUSION

The developed QSPECT package allows absolute CBF and CVR to be estimated in routine clinical studies. This multicenter study has demonstrated the applicability of QSPECT for a variety of clinical settings and equipment. Results from the studies suggest that a change of approximately 10% or 5 mL/min/100 g can be readily detected in follow-up studies. The graphical user interface for easily controlling the in-built sophisticated programs and tools ensures that routine use does not require dedicated support from scientific or computing staff. The package is now successfully used in over 130 institutions in Japan, and more than 25,000 patient studies have been analyzed with the QSPECT package.

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Design and characterization of a polymeric MRI contrast agent based on PVA for *in vivo* living-cell tracking

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A novel water-soluble MRI contrast agent for *in vivo* living cell tracking was developed. Unlike the conventional *in vivo* cell tracking system based on superparamagnetic iron oxide beads, the newly developed contrast agent is eliminated from the body when the contrast agent exits the cells upon cell death, which makes living cell tracking possible. The contrast agent is composed of gadolinium chelates (Gd–DOTA) and a water-soluble carrier, poly(vinyl alcohol) (PVA), which is known to interact with cells and tissues very weakly. Since the Gd–PVA was not taken up by cells spontaneously, the electroporation method was used for cell labeling. The delivered Gd–PVA was localized only in the cytosolic compartment of growing cells with low cytotoxicity and did not leak out of the living cells for long periods of time. This stability may be due to the weak cell-membrane affinity of Gd–PVA, and did not affect cell proliferation at all. After cell labeling, signal enhancement of cells was observed *in vitro* and *in vivo*. These results indicate that Gd–PVA can visualize only the living cells *in vivo* for a long period of time, even in areas deep within large animal bodies. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: MRI; cell tracking; intracellular delivery; cell transplantation; Gd chelate

1. INTRODUCTION

Over the past decade, there has been increasing interest in developing cell transplantation therapy (1–3) for various diseases such as ischemic limbs (4), infarcted myocardium (5,6) and diabetic retinopathy (7). In particular, the transplantation of autologous cells such as bone marrow- or fat tissue-derived mesenchymal stem cells is much safer than heterologous transplantation in terms of rejection, and is promising in clinical use. However, the mechanism of cell transplantation therapy remains a matter of debate. One possible mechanism is the differentiation of transplanted cells into functional cells, and another is the paracrine effect due to the produced cytokines (8). Moreover, even the engraftment ratio and survival period of the transplanted cells remain unclear. A general method of analyzing the transplanted cells, such as immunostaining, cannot be used for autologous cell transplantation because there is no phenotypic difference between transplanted cells and host cells. In recent years, then, noninvasive tracking systems for cell transplantation are attracting a great deal of attention (9,10).

Optical imaging methods using fluorescence- or bioluminescence-labeled cells have been studied extensively (11,12). Recently, green fluorescent protein (GFP)-transgenic animal or GFP-positive cells have become widely available and have been easily analyzed using various *in vivo* optical imaging instruments. However, since optical lights can penetrate tissues less than 10 mm in the case of fluorescence and 30 mm in the case of bioluminescence, only mice or rats can be used in this system (13). Therefore, cell transplantation model systems cannot be used for various diseases in large animals (14–16). In addition, the resolution is low, and the transplanted cells can be detected as large circles in small animals (13).

In contrast, magnetic resonance imaging (MRI) is a more promising system because of its high resolution, its absence of limitations on animal size and its noninvasiveness. In order to detect the transplanted cells in host tissues using MRI, cells should be labeled with contrast agents. In the past 15 years, superparamagnetic iron oxide particles (SPIO) have been studied as a means of labeling cells because of their high sensitivity (17,18). SPIO are superior to other contrast agents in terms of the detection of cells. Rice *et al.* reported the homing phenomena of adipose-derived stem cells in cerebral infarction (19). Stuckey *et al.* reported the monitoring of bone marrow stromal cells in the infarcted heart (20). Targeted cells were usually labeled with SPIO by the endocytosis mechanism or by using gene-transfection agents. However, in long-term tracking of cells, one of the problems with this system is the fate of SPIO which leaks out of

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cells (free SPIO) due to exocytosis or cell death. Additionally, SPIO that has undergone intracellular uptake is slowly digested, releasing its iron. The free SPIO remains in the body and continues to show MR contrast, creating the potential for misunderstanding (21). Amsalem *et al.* reported that the observed MRI signals after transplantation of SPIO-labeled MSCs were not attributed to transplanted cells but to cardiac macrophages which took up the released SPIO from transplanted cells (22). Li *et al.* reported that SPIO undergoing cell death was internalized by macrophages or remained in the local tissue (23).

The most important part of cell tracking is to track only the living cells. In the present study, a novel water-soluble contrast agent was designed and an effective intracellular delivery system was established. Gd–DOTA (1,4,7,10-tetraazacyclo-dodecane-*N,N',N'',N'''*- tetraacetic acid) was conjugated to a bioinert and highly water-soluble polymeric carrier, poly(vinyl alcohol) (PVA). PVA is known to circulate for a long period of time in the blood stream *in vivo* because of its very weak interaction with the blood cells, macrophages or tissues. It was reported that the amount of PVA ingested by macrophages was much less than that of bovine serum albumin (24). The conjugates would be expected to be eliminated from the tissues without being ingested by macrophages when they are outside of the dead cells. The intracellular delivery system of the conjugates was established using an electroporation system, and the cytotoxicity, intracellular stability, body distribution and MR-imaging ability of the contrast agent were studied *in vitro* and *in vivo.*

2. RESULTS

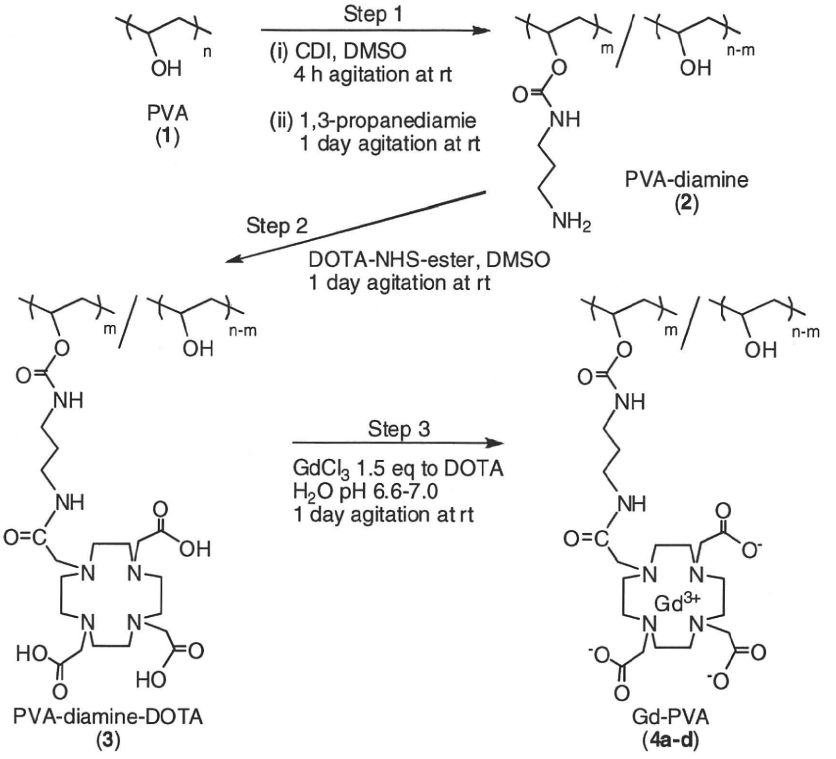
2.1. Synthesis of Gd–PVA (*4a–d*)

Conjugates **4a–d** were synthesized in three steps using PVA with a molecular weight of 74 800 (**1**) as shown in Scheme 1. The structure of conjugates **4a–d** was confirmed by ^{1}H-NMR spectroscopy and their characteristics are summarized in Table 1. At step 1, the introduction ratios of diamine ($m/n \times 100$ in Scheme 1) were 13.2, 7.5, 3.6 and 12.9%, respectively. At step 2, DOTA-NHS-ester was completely reacted with free NH_2 groups on **2** because the peak of 2.79 ppm had disappeared. These polymers were soluble in water and DMSO and insoluble in acetone, toluene and tetrahydrofuran. The Gd (III) content of the conjugates (**4a–d**) was analyzed by inductively coupled plasma atomic emission spectroscopy. To observe the cell labeling efficiency and the intracellular distribution of the conjugates, Gd–PVA labeled with fluorescence (**4d**) was synthesized. MR imaging of labeled cells was carried out after confirming the cell uptake of **4d** with fluorescent microscopy. By contrast, the cytotoxicity assay was performed using **4b** without FITC because the wavelength of FITC overlapped with that of the WST assay.

The increase of the relaxivities (R_1) of **4a–d** with the increased introduction ratio of DOTA may be due to an increased rotational correlation and constructive restriction of motion. A maximum reflexivity value of 7.1 $mM^{-1}s^{-1}$ was observed at 13.2 mol% (**4a**). All of the relaxivities of **4a–d** were higher than that of clinically used Gd–DTPA (5.1 $mM^{-1}s^{-1}$), suggesting that each conjugate can be used as an effective contrast agent.

2.2. *In vitro* T_1-weighted MR measurements of polymer solutions

Figure 1 shows the MR images of **4d** solutions with different concentrations at 4.7 T. The T_1-weighted MRI signal of the **4d** solution increased with the increased polymer unit concentration. Significant contrast enhancement was seen over 0.2 mM. To achieve cell imaging, it is necessary to introduce the contrast agents at sufficient concentrations in the cells.



Scheme 1.

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Table 1. Synthesis of **4a–d** with different contents of gadolinium chelates

	Introduction ratio of DOTA[a] (mol%)	Mn[b] ($\times 10^5$)	M_w/M_n[b]	Gd[c] (wt%)	Gd/DOTA (mol%)	FITC label	R_1 (mM^{-1} s^{-1})
4a	13.2	1.6	1.1	12.0	70.0	–	7.1
4b	7.5	1.1	1.2	9.2	69.1	–	6.2
4c	3.6	1.2	1.2	5.8	67.0	–	6.2
4d	12.9	—	—	9.3	53.9	+	7.0

[a]Scheme 1, $m/n \times 100$.
[b]Determined by size exclusion chromatography using 0.25 mM phosphate buffer as eluent with polystyrene standards.
[c]Determined by inductively coupled plasma atomic emission spectroscopy measurement.

2.3. Cytotoxicity of Gd–PVA to NIH-3T3 cells and cell labeling by electroporation

Gd–PVA **4b** was used for a cytotoxicity assay since FITC introduced to **4d** obstructs the accurate WST-1 assay. The viability of NIH-3T3 cells in the presence of **4b** was not affected even at high concentrations (10 mM; polymer unit concentration in culture medium) for up to 3 days (see Supplementary Information). The low affinity of PVA (24) might suppress the interaction of Gd–PVA with the cell membrane and decrease the cytotoxicity. In fact, weak interaction was demonstrated by a simple experiment as follows. Compound **4d** was added to the culture medium of NIH-3T3 cells, and the cells were incubated for 1 h. After washing with PBS three times, no fluorescence induced by **4d** was observed, indicating that **4d** was unable to attach to the cell membrane or enter the cells spontaneously.

To deliver such a bio-inert substance into cells, we selected an electroporation method that is mainly used to transfect DNA into cells. Since this method can introduce a large amount of polymeric substances into any kind of cells nonspecifically with low cytotoxicity, it is suitable for labeling various cells including established cell lines, somatic stem cells, or even embryonic stem cells for cell transplantation (25,26). When electroporation was carried out, the concentration of Gd–PVA in culture medium was set to 10 mM (polymer unit concentration) based on the result of the cytotoxicity assay.

Figure 2 shows bright field and fluorescent photomicrographs of NIH-3T3 cells 3 days after electroporation with **4d**. Almost all cells were labeled efficiently, and the intracellular **4d** was interestingly located only in the cytosolic compartment of NIH-3T3 cells even after cell proliferation. This intracellular distribution pattern is different from that for endocytosis, which is made from bright dots.

The stability of Gd–PVA in NIH-3T3 cells was assessed by measuring the total fluorescence intensity of the growing NIH-3T3 cells with time. The number of Gd(III) molecules in one cell calculated from the fluorescence intensity was 7.3×10^8 per cell just after electroporation. Cells were cultured for a given period of time without subculture and then lysed. Before the cells were lysed, they were washed by PBS sufficiently to eliminate any **4d** leaching from them. Figure 3 represents the total fluorescence intensity of **4d** in NIH-3T3 cells (solid circle) and cellular proliferation rates (open circle). Fluorescence derived from **4d** in cells showed no significant change over 10 days, and the labeled cells grew well. These results show that **4d** can remain in the cytosolic compartment stably for a long period of time without having any effect on cell proliferation.

2.4. *In vitro* T_1-weighted MR measurements of the labeled NIH-3T3

Figure 4a shows an MR image of the NIH-3T3 cell suspensions at 4.7 T. Compound **4d**-labeled NIH-3T3 cell suspension, non-labeled NIH-3T3 cell suspension and cell-free and Gd-free medium were left at rest for 1 day to allow the cells to be precipitated to the bottom of the test tube. Clear signal enhancement in tube 1 at slice B passing through the precipitated cells was seen. On the other hand, no signal was observed in tube 1 at slice A, which indicates that **4d** did not leak out of the cells and that **4d** in cells gives sufficient MR contrast irrespective of the small amount of free water in the cells

To examine the cell density dependence of signal enhancement, we next acquired MR images of **4d**-labeled NIH-3T3 cells at different densities in agarose gel, which was used to fix the transplanted cells in the experiment involving the injection of cells into a rat (Fig. 4b). MRI can depict at least 3.5×10^6 NIH-3T3

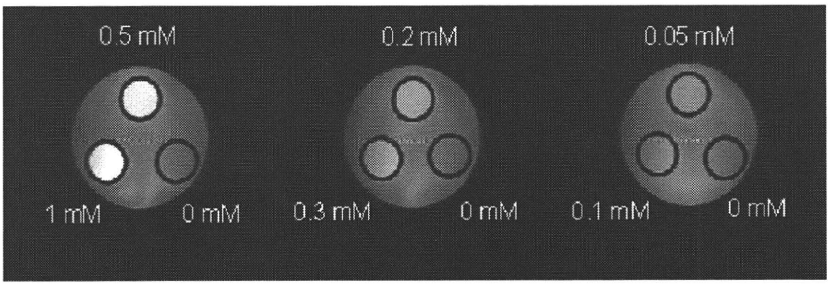

Figure 1. *In vitro* T_1-weighted MR measurements of **4d** in water at 4.7 T at the concentrations of 0, 0,05, 0.1, 0.2, 0.3, 0.5, and 1.0 mM. Three test tubes containing different concentrations were fixed vertically. A horizontal section was scanned. These images were acquired using a 2 D spin echo sequence with a TR of 2000 ms and a TE of 16 ms. These images were displayed using the same window level and window width.

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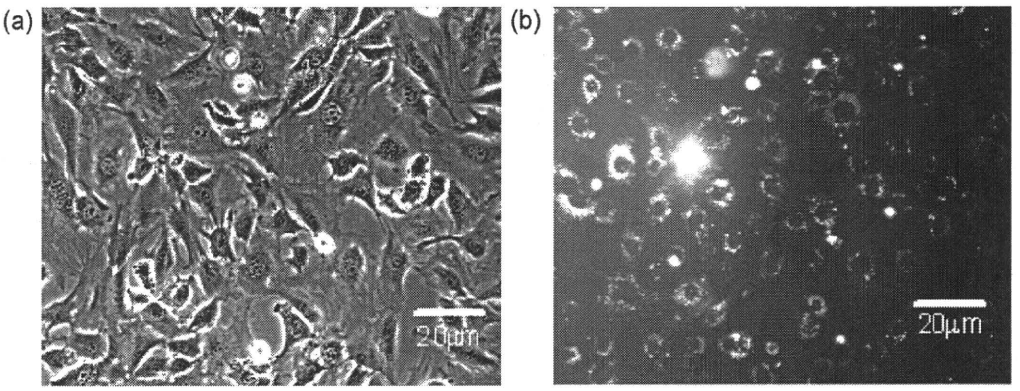

Figure 2. (a) Phase image and (b) fluorescent image of NIH-3T3 cells labeled with **4d** (FITC-Gd-PVA) at 3 days after electroporation. After electroporation, cells were washed three times by PBS. The bright ring forms showed cytosolic compartments in the fluorescent image. The scale bar represents 20 μm.

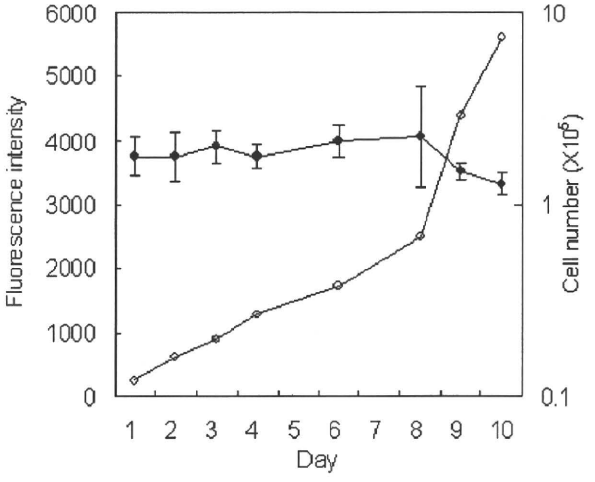

Figure 3. Changes in fluorescence intensity of **4d** existing in total NIH-3T3 cells in culture (solid circle) and the number of cells (open circle) measured over the course of the 10 days following electroporation. Fluorescence intensity is proportional to the amount of **4d** in total cells.

cells. The number of cells transplanted to the rat ischemic hind limb model (27) or infarcted myocardium swine model (28) was 1×10^7 or 5×10^7, respectively. The sensitivity shown in Fig. 4 revealed that our imaging agent would surely be useful for tracking this range of transplanted cells *in vivo*. Future studies should focus on high labeling efficiency at higher concentrations of **4d** using electroporation or another method.

2.5. *In vivo* fate of free SPIO and free Gd–PVA

To detect the living cells, contrast agents present outside of the labeled cells (free contrast agent) after cell death should be eliminated from the transplantation site. Solutions of **4d** and SPIO injected into the tissue were used as the model for free contrast agents. Solutions of **4d** and SPIO were directly injected into rat femoral muscles, and on days 0, 3 and 6, the MR image was analyzed (Fig. 5). Representative slices are shown in Fig. 5. The bright signal attributed to **4d** weakened rapidly and was observed only slightly on day 3. In contrast, the dark signal due to SPIO remained in the same area and was clearly observed even 10 days after the injection. The same tendency was observed in the other slices. SPIO-derived contrast several days after injection may be attributed to the phagocytes engulfing the injected SPIO, as has been previously reported (22,23). Furthermore, the time courses of the contrast-to-noise ratio (CNR) and the volume of the contrast-enhanced region were evaluated (Fig. 6). For SPIO, the CNR and the volume of the contrast-enhanced region showed no significant decrease over the course of 13 days. In contrast, these same parameters decreased rapidly when **4d** was used. Signal enhancement was observed in only one out of three rats at 4 days after injection. Therefore, the data of **4d** at 4 days have no error bar. Signal

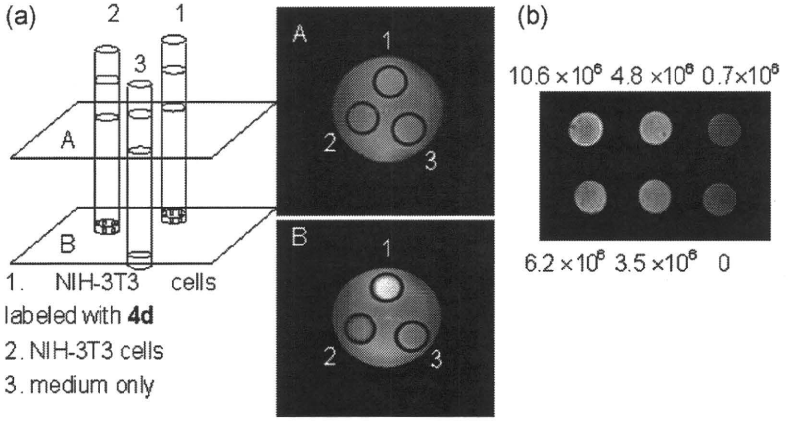

Figure 4. (a) *In vitro* T_1-weighted MR measurements of **4d**-labeled NIH-3T3 cells (tube 1), unlabeled NIH-3T3 (tube 2), and medium (tube 3) at 4.7 T. (b) *In vitro* T_1-weighted image of different numbers of cells labeled with **4d** suspended in 100 μl agarose gel.

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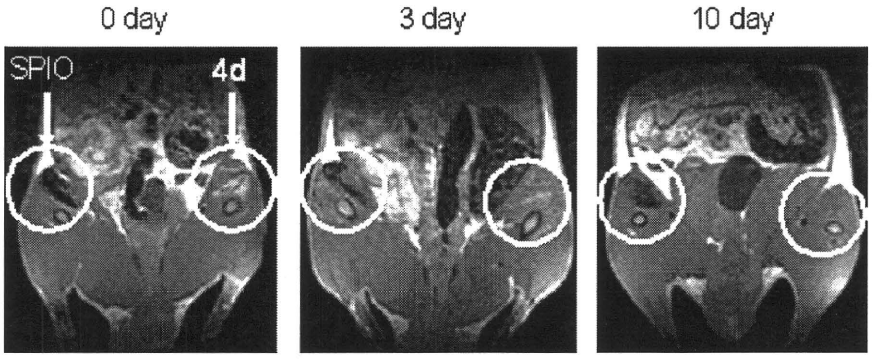

Figure 5. *In vivo* MR measurements after the injection of **4d** solution and SPIO solution into rat femoral muscle at 1.5 T. These images showed the slices passing though the injection site. These images were obtained with a TR of 1500 ms and a TE of 9 ms (FOV, 4 × 8 cm; matrix, 128 × 256; slice thickness, 1 mm; slice gap, 0 mm; number of slices, 35).

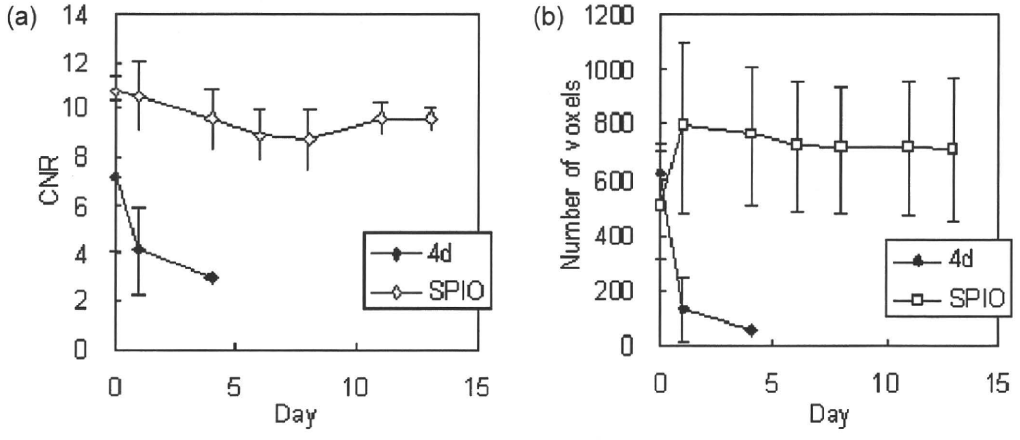

Figure 6. The time course of (a) the contrast-to-noise ratio (CNR) and (b) the number of voxels in the region where bright or dark signals due to contrast agents were observed. Contrast enhancement due to **4d** and SPIO was assessed using T_1- (TE 9 ms, TR 500 ms) and T_2- (TE 20 ms, TR 3 s) weighted images, respectively. The other scanning parameters were the same as in Figure 5. Three rats were examined and treated in the same manner as in Figure 5. CNR was calculated as $(\pi/2)^{1/2}|S_1\text{-}S_2|/S_{air}$, where S_1, S_2 and S_{air} were the mean intensities in the contrast-enhanced region, muscle and air, respectively.

enhancement due to **4d** disappeared completely in all rats at 6 days after injection. These data showed the rapid clearance of Gd–PVA from muscle and the long-term retention of SPIO in muscle. Yamaoka *et al.* reported that the half-life period of radio-labeled PVA (molecular weight of 74 800) after i.m. injection was about 10 h (38). As shown in Fig. 6, the half-life period of free Gd–PVA from the tissue was about 10 hs, which was almost the same as that of PVA. This result suggested that free Gd–PVA behaved like free PVA without interacting with macrophages *in vivo*. It can then be considered that the MR contrast of Gd–PVA is attributable to the living cells *in vivo*.

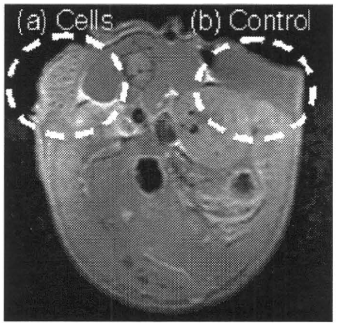

Figure 7. Preliminary *in vivo* T_1-weighted MR measurements of **4d**-labeled NIH-3T3 cells implanted in mice subcutaneously at 2 T. These cells were fixed in agarose gel. (a) 2×10^7 of **4d**-labeled cells suspended in 200 μl agarose gel (b) 200 μl agarose gel only. T_1-weighted images were acquired using a 2D spin echo sequence with a TR of 2000 ms and a TE of 9 ms (FOV, 3 × 6 cm; matrix, 128 × 256; slice thickness, 1 mm) at room temperature.

2.6. Preliminary *in vivo* MR imaging of transplanted NIH-3T3 cells

Figure 7 shows an MR image of a rat that received subcutaneous transplantation of 2×10^7 **4d**-labeled NIH-3T3 cells entrapped in agarose gel and cell-free gel (control) at each side of the back. In this preliminary MR imaging, we used undegradable agarose gel to evaluate the MRI contrast at a known density of cells. Strong contrast enhancement was observed at the area where labeled NIH-3T3 cells were transplanted, while the control gel revealed a dark shadow. These results indicate that transplanted cells can be detected *in vivo* at a cell density of 10^7 cells per 0.1 ml.

3. DISCUSSION

Our goal was to track only the living cells *in vivo* for a long period of time. To this end, an MRI contrast agent with adequate

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characteristics for cell labeling and delivery system into the cells is a key factor. Cell labeling using SPIO as a contrast agent was reported in detail by Engberink *et al.* in 2007 (29). They cocultured human monocytes with SPIO suspension at a concentration of 1.0 mg Fe ml^{-1} for 0–6 h. Incubation with SPIO resulted in effective cell labeling by endocytosis, nonspecifically. The detection limit was 0.5×10^6 labeled cells per 250 μl on a 4.7 T MRI scanner. SPIO permits the detection of a small number of cells because of its high sensitivity. In general, however, endocytosed substrates would be exocytosed over time. The MR contrast obtained after SPIO-labeled cell transplantation was not attributed to the transplanted cells but to the macrophages that engulfed the free SPIO (22,23). In this study, MR imaging data for SPIO solution in femoral muscle showed that, even at 10 days after injection, SPIO still remained. These data suggested that SPIO is less suitable for long-term cell tracking. To track the transplanted cells for a long period of time, the labeling agent released upon cell death should be eliminated from the tissue.

Since we found that low-molecular-weight Gd-chelates cannot remain in cells stably (data not shown), water-soluble conjugates of Gd-chelates and a bio inert water-soluble carrier were designed. The characteristics of Gd-containing conjugates including the body distribution pattern are affected by the nature of the carrier polymer. The water-soluble contrast agent is expected to be eliminated from the body once it exits the cells if a truly bio-inert carrier molecule is selected. To track only the living cells, the contrast agents should be designed to be different from the conventional water-soluble imaging agent for vascular inflammation imaging or vascular imaging (30–36).

We selected PVA in this experiment as the carrier material for long-term living cell tracking. Selecting nondegradable PVA as the carrier enabled us to evaluate the potential of the contrast agent in intracellular distribution or in cell tracking for a long period of time. The body distribution of various polymeric carriers has been extensively studied (37,38). Among these carriers, PVA has various advantages as a candidate for use in the biomedical and pharmaceutical fields. Some of these advantages include its characteristics of water solubility, nontoxicity and noncarcinogenicity. The half-life of Gd–PVA was longer than those of other polymers such as dextran, pullulan and gelatin because of an insignificant interaction with macrophages and blood cells (24). This weak interaction with various cells is believed to be responsible for the high hydrophilicity of PVA. Since we proposed novel contrast agents in the present study that would not exit the cells for long-term cell tracking, this weak interaction with the cell membrane was considered to be an advantage.

In the present study, we chose electroporation as a method for delivering Gd–PVA into cells in order to establish a method that is applicable to a variety of cells such as stem cells and primary cells. The material delivery efficiency into cells via nonspecific endocytosis or receptor-mediated encodytosis is probably affected by the cell type. Interestingly, Gd–PVA delivered into cells was localized only in the cytosolic compartment even after cell proliferation (Fig. 2), although the reason for this remains unclear.

One possible issue in living cell tracking, although unlikely to occur, is the uptake of dying cells labeled with **4d** by tissue macrophages that remain in the tissue. To study this possibility, it is necessary to perform an experiment using cells in different states (viable, dying and dead). However, it is difficult to control the states of transplanted cells. We are considering evaluating the effect of macrophages on the fate of Gd–PVA by transplanting irradiated cells with sublethal doses or by xenografting Gd–PVA-labeled cells.

Long-term cell tracking will be feasible due to the high stability of Gd–PVA in cells for a long period of time (Fig. 3). In contrast to SPIO, the free Gd–PVA will be eliminated from the tissue (Fig. 5) when the transplanted cells burst upon cell death. The imaging of only the living cells might be achieved using Gd–PVA.

4. CONCLUSION

The novel MRI contrast agents composed of PVA and Gd showed high relaxivity and low cytotoxicity. The growing rate of NIH-3T3 cells was not affected by the intracellularly delivered Gd–PVA. Furthermore, Gd–PVA was retained stably in cells for at least 10 days. The *in vitro* T_1-weighted MR measurements using NIH-3T3 cells revealed that cells could be visualized under MRI. This *in vivo* study demonstrates for the first time that Gd–PVA has high applicability as a novel contrast agent for tracking only living cells.

5. MATERIALS AND METHODS

5.1. Materials

PVA (M_w: 74,800, degree of saponification 98%) was a kind gift from Kuraray Co. Ltd (Okayama, Japan). 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(*N*-hydroxysuccinimidyl ester) (DOTA-NHS-ester) was purchased from Macrocyclics (Dallas, TX, USA). FITC-NHS-ester was purchased from Invitrogen (Eugene, OR, USA). Gadolinium chloride ($GdCl_3$) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Resovist was purchased from Nihon Schering (Osaka, Japan). Other reagents and solvents were commercially available and used as received.

5.2. Synthesis of Gd–PVA

The synthetic route and structure of polymeric contrast agents with different introduction ratios of Gd are shown in Scheme 1. A mixture of PVA (**1**; 0.44 g, 10 mmol in monomer unit concentration) and carbonyl diimidazole (5, 7.5, and 10 mmol) was stirred in 80 ml of anhydrous dimethylsulfoxide (DMSO) at room temperature under a nitrogen atmosphere for 4 h. Then, 1,3-propanediamine (50, 75, and 100 mmol) was added to the mixture, further stirred at room temperature for 1 day, and dialyzed with Spectra/Pore membrane (cut-off molecular weight = 1×10^4; Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) in distilled water three times. The remaining solution was lyophilized to give **2**.

^{1}H NMR (D_2O): $\delta = 4.92$ (br, CH_2CHO), 3.92 (br, CH_2CHOH), 3.10 [br, $C(=O)NHCH_2$], 2.79 (br, CH_2NH_2), 1.57 (br, $CHCH_2$, br, $CH_2CH_2CH_2$). The introduction ratios were calculated as the ratio of the integrals of the peaks at 2.79 and 1.57 ppm.

PVA-diamine was reacted with DOTA-NHS-ester (NH_2 of FITC-PVA-diamine: DOTA-NHS-ester = 1:1.5) in 80 ml of anhydrous DMSO at room temperature for 1 day under a nitrogen atmosphere. The reaction mixture was dialyzed in distilled water three times, and lyophilized to give PVA-diamine-DOTA (**3**).

^{1}H NMR (D_2O): $\delta = 5.07$ (br, CH_2CHO), 4.06 (br, CH_2CHOH), 3.86 [br, $C(=O)CH_2N$)] 3.51 [br, $NCH_2C(=O)OH$], 3.24 [br, $C(=O)NHCH_2$, br, CH_2CH_2N], 1.69 (br, $CHCH_2$, br, $CH_2CH_2CH_2$).

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The solution of **3** was then treated with the dropwise addition of 1.5 mole equiv. of gadolinium chloride to the DOTA while stirring. The pH was maintained between 6.6 and 7.0 with 1 M NaOH solution and stirred for an additional 24 h at room temperature. The reaction mixture was dialyzed in distilled water three times and lyophilized to give Gd–PVA (**4a–d**).

For labeling Gd–PVA with FITC, PVA–diamine was mixed with a small amount of FITC–NHS–ester (NH_2 of **2**: FITC–NHS–ester = 1: 8×10^{-5}) and stirred in 80 ml of anhydrous DMSO at room temperature for 1 day under a nitrogen atmosphere. The reaction mixture was dialyzed, lyophilized to give FITC-PVA-diamine and subjected to the DOTA reaction as shown in Scheme 1.

5.3. Measurements

^{1}H-NMR spectra were recorded on a 300 MHz NMR spectrometer (Gemini2000/300; Varian Inc., CA, USA) with a sample concentration of 8 mg per 800 μl. Size exclusion chromatography analysis was carried out using Shimadzu Gel Permeation Chromatography System apparatus equipped with a refractive index and UV detectors under the following conditions: TSKgel G6000PWXL and G3000PWXL columns and 0.067 M PBS eluent at a flow rate of 0.3 ml min^{-1} at 40°C (Tosoh, Tokyo, Japan) with a sample concentration of 1 mg per 100 μl. The concentration of the paramagnetic species [Gd(III)] was measured by inductively coupled plasma atomic emission spectroscopy (model 7510, Shimadzu Co., Kyoto, Japan).

5.4. Relaxivity of conjugated Gd at 7.1 T

Solvent longitudinal relaxation times (T_1) in the aqueous solutions of the gadolinium conjugate were measured at different concentrations of gadolinium conjugate using a mixture of distilled water (0.625%) and deuterium oxide (99.375%) as a solvent. All measurements were performed on a 300 MHz (7.1 T) NMR spectrometer (Gemini2000/300; Varian Inc., CA, USA) using an inversion recovery technique with 19 inversion times (*TI*) ranging from 1 to 5000 ms at ambient temperature (25°C) with a sample concentration of 8 mg per 800 μl. A typical pulse width of 180° pulse was 19 μs. T_1 values were estimated using least-squares fitting of the signal intensities measured at 19 *TI* values in an exponential fashion. The relaxivity of each gadolinium complex was determined by a linear regression of the $1/T_1$ vs the gadolinium complex concentration.

5.5. Cell culture

NIH-3T3 cells were used for evaluating the cytotoxicity, cell labeling potential and imaging efficiency of the Gd–PVA. They were grown in Dulbecco's modified Eagle's medium (DMEM-LG) supplemented with 10% bovine calf serum, 100 units ml^{-1} penicillin, and 100 units ml^{-1} streptomycin at 37°C, 10% CO_2 atmosphere.

5.6. Cytotoxicity assay

NIH-3T3 cells (1×10^4 cells per well) were seeded in a 96-well culture plate and cultured overnight. Varying concentrations (polymer unit concentrations of 10 nM to 10 mM) of **4b** were added to each well. At the indicated time points, the number of cells was measured by WST-1 assay according to the manufacturer's protocol (Takara Shuzo, Otsu, Japan). Briefly, cells were washed with PBS three times, and the culture medium (100 μl) was added to each well. Ten microliters of WST-1 {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate} solution was added to each well, and the plates were incubated for 30 min. The absorbance at 450 nm was measured on a microplate reader (Model 550, Bio-Rad Laboratory Co., Tokyo, Japan).

5.7. Cell labeling by electroporation

NIH-3T3 cells were cultured in a 6 cm diameter Petri dish at a concentration of 5×10^5 cells per dish in DMEM-LG for 1 day. An arbitrary amount of **4d** was added to the culture medium, and electrical pulses were applied to cells using a CUY-21 electroporator (CUY-21; NEPPA GENE, Tokyo, Japan). Rectangular electrical pulses (field strength 300 V cm^{-1}, number of pulses 10, pulse duration 5 ms) were applied to cells using two parallel electrodes with a 5 mm gap. Cells were incubated for 1 h and washed with PBS twice.

5.8. Stability of 4d in cells

To determine whether **4d** molecules stay in NIH-3T3 cells for a long period of time, the labeled cells (1×10^4 cells) were seeded in a 6 cm diameter Petri dish and cultured over 10 days without a subculture. The time course of the fluorescence intensity for the cultured cells was measured as follows. Before each measurement, cells in one dish were washed three times with PBS to eliminate the free **4d** from the cells and lysed in 1 ml lysis buffer [25 mM tris (pH 7.8), 2 mM dithiothreitol, 2 mM 1,2-diaminocyclohexan-*N,N,N',N'*-tetraacetic acid, 10% glycerol, 1% Triton X-100]. After 1 h incubation at 37°C, the fluorescence intensity of the cell lysates was measured with a spectrofluorometer (excitation 430 nm, emission 540 nm, Wallac 1420 ARVOsx, Perkin-Elmer Life Sciences, Boston, MA, USA). The time course of the fluorescence intensity represented the stability of **4d** in the cells. At the same time, the number of cells in each dish was counted. In addition, the amount of **4d** delivered into each cell by electroporation was calculated using the standard curve of fluorescence intensity.

5.9. MR imaging of Gd–PVA solution at 4.7 T

MR images of **4d** aqueous solutions were obtained on a 200-MHz (4.7 T) NMR spectrometer (Apollo; Tecmag Inc., TX, USA) equipped with a gradient system (Jeol Ltd, Tokyo, Japan; maximum gradient strength 20 mT m^{-1}; slew late 50 mT m^{-1} ms^{-1}) using a saddle coil with an inner diameter of 47 mm. Aqueous solutions with different concentrations (0.05, 0.1, 0.2, 0.3, 0.5 and 1 mM) of polymer unit were prepared. Three test tubes with different concentrations were fixed vertically. A horizontal section was scanned. T_1-weighted images of the samples were acquired using a 2D spin echo sequence with a repetition time (*TR*) of 2000 ms and an echo time (*TE*) of 16 ms. Taking the long T_1 of the water observed in the 1.5 T machine into account, *TR* was greater in comparison to that for general T_1-weighted images. We used the minimum possible *TE* to minimize the T_2 relaxation effect. Other scanning parameters were as follows: field of view (FOV), 6 × 6 cm; matrix, 256 × 256; slice thickness, 1 cm.

5.10. MR imaging of NIH-3T3 cells *in vitro*

MR measurements of labeled cells were performed using the same scanner and the same parameters as in the imaging of **4d** solutions. Cells labeled with **4d** by electroporation were trypsinized, centrifuged and resuspended in test tubes (75 mm

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long, 10 mm in diameter) at 7×10^6 cells in 2 ml of complete DMEM. The test tubes with labeled cell suspensions were allowed to settle for 1 day to allow the cells to be precipitated before MR imaging. A test tube with unlabeled cell suspensions was also prepared in the same manner. In addition, a test tube with cell-free pure medium was prepared. The three test tubes prepared were arranged as shown in Fig. 4(a). Scanned slices were positioned so that they pass through the cell pellet part (slice B in Fig. 4a) or the solution part (slice A in Fig. 4a).

The cell density dependence of signal enhancement was examined as follows. Different numbers of labeled cells were suspended in 100 μl of agarose solution at the concentration of 2 wt% and cooled to be gelated. The MR imaging data of these mixtures were collected by a 1 T compact MR imaging system with a permanent magnet (MRmini, Dainippon Sumitomo Pharma, Osaka, Japan) with a *TE* of 9 ms and a *TR* of 1500 ms (FOV, 3 × 6 cm; matrix, 128 × 256; slice thickness, 3.7 mm).

5.11. *In vivo* fate of free SPIO and free Gd–PVA

The clearance of **4d** and SPIO after intramuscular injection was investigated in male rat F344. The rat was anesthetized by inhalation anesthesia (1.5% isoflurane). Solutions of **4d** (Gd 0.8 μmol per 50 μl water) and carboxydextran-coated SPIO, Resovist™ (Fe 0.8 μmol per 50 μl water, Bayer, Osaka, Japan) were injected into the left and right femoral muscles, respectively, using a 29 G needle. Whole inferior limbs of the animal were scanned at 0, 3 and 10 days after injection on a 1.5 T compact MR imaging system. These images were obtained with a *TR* of 1500 ms and a *TE* of 9 ms (FOV, 4 × 8 cm; matrix, 128 × 256; slice thickness, 1 mm; slice gap, 0 mm; number of slice, 35).

For the time course of the CNR and the number of voxels in the region, whole inferior limbs of the animal were scanned at 0, 1, 4, 6, 8, 11 and 13 days after injection on a 1.5 T compact MR imaging system. These images were obtained with a *TR* of 500 ms and a *TE* of 9 ms, and with a *TR* of 3000 ms and a *TE* of 20 ms (FOV, 4 × 8 cm; matrix, 128 × 256; slice thickness, 1 mm; slice gap, 0 mm; number of slices, 35). CNR was calculated as $(\pi/2)^{1/2}|S_1-S_2|/S_{air}$, where S_1, S_2 and S_{air} were the mean intensities in the contrast-enhanced region, muscle and air, respectively.

5.12. Preliminary MR imaging of transplanted NIH-3T3 cells

In vivo cell tracking was preliminarily performed in male Balb/c mice. These mice were anesthetized for imaging with the use of a general inhalation anesthesia (1.5% isoflurane) and were allowed to breathe spontaneously during preparation and scanning. NIH-3T3 cells labeled with **4d** (2×10^7 cells) were embedded in 2 wt% agarose gel (200 μl) and transplanted to the mice subcutaneously. MR images were obtained using a 2 T compact MR imaging system with a permanent magnet. T_1-weighted images were acquired using a 2D spin echo sequence with a *TR* of 2000 ms and a *TE* of 9 ms (FOV, 3 × 6 cm; matrix, 128 × 256; slice thickness, 1 mm) at room temperature.

6. SUPPORTING INFORMATION

Supporting information can be found in the online version of this article.

Acknowledgements

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特集 ここまできた神経画像

核医学的測定法の進歩

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PET 装置は検出器の精細化で空間解像度の上昇が進み，立体撮像によって高感度化が実現した．SPECT においては吸収と散乱線を正確に補正する画像再構成プログラムの利用によって，定量化と標準化が可能になった．このようななかで，従来よりも一歩踏み込んだ脳神経核医学画像の定量評価が可能になる．複数モダリティイメージングに加えて，調節機能などの診断は今後の課題である．

Key Words
機能画像，マルチモダリティ，マルチトレーサ，時間解像度

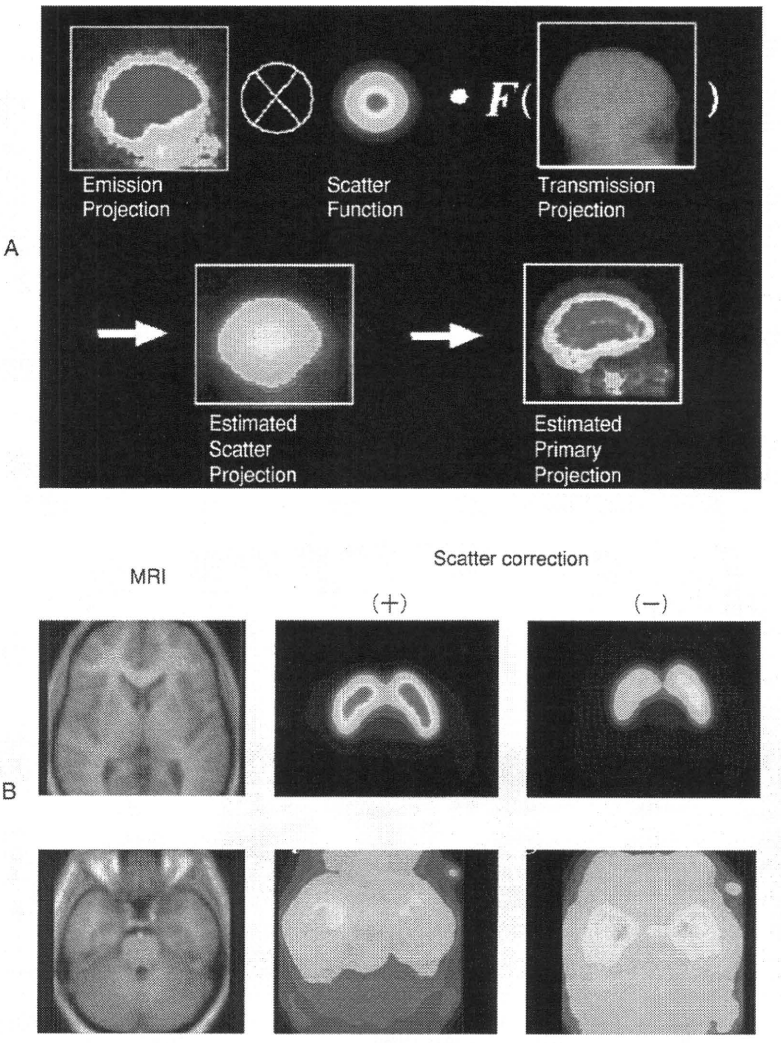
はじめに

PET が誕生してから 30 年以上の技術蓄積の結果，検出器の改良は空間解像度の一桁向上に，多断層化と立体計測技術の普及は感度の二桁上昇に貢献した．SPECT においては複数検出器やコリメータの進歩，さらに画像再構成理論の改善によって定量精度改善がなされた．複数モダリティの融合画像処理技術や，新しい画像再構成理論の整備と実用化も進み，神経画像の高精細化に貢献する．本稿では，新しい要素技術とその効果について概説する．

1 PET

PET 装置の空間解像度を決定するのはシンチレーション結晶の大きさであり，小型ブロック検出器の開発が高解像度化に貢献してきた．深さ情報（depth-of-interaction：DOI）を検出する検出器も実用化された．立体計測（三次元，3D）は完全に定着し測定感度は大きく改善し，1%を超えるに至った．臨床 PET 装置の空間解像度は概ね 4 mm 程度にまで向上した．画像再構成プログラムはさらに改良が進み，吸収補正や散乱線補正に加えて空間解像度の補正がなされるようになり，空間解像度は 2 mm 程度になったとされる．高性能な放射線検出シンチレータの実用化がなされ，また同時計測の時間分解能（coincidence window）が短くなった．これは画質劣化の最大の要因であった偶発同時計数の減少に貢献する．一部のメーカーでは time-of-flight 処理（同時計測信号の時間差より線源の場所を限局する機構）も搭載される．

現在の課題は，膨大な情報の高速処理技術の開発にあるといえる．PET では検出器総数の自乗に比例した計測線（line-of-response：LOR）の信号を扱う．典型的には



図❶ 定量的 SPECT 画像再構成における散乱線補正の効果
（A：Iida H *et al*, 1998[2]，B：Fujita M *et al*, 2004[4]より引用）
A：散乱線補正法の例．吸収減弱プロジェクションを利用した方法はノイズの高揚がなく良好な画像を提示する．B：I-123 標識 epidepride の結合能画像における散乱線補正の効果．散乱線補正は画像のコントラストを上昇させ，定量解析には不可欠である．

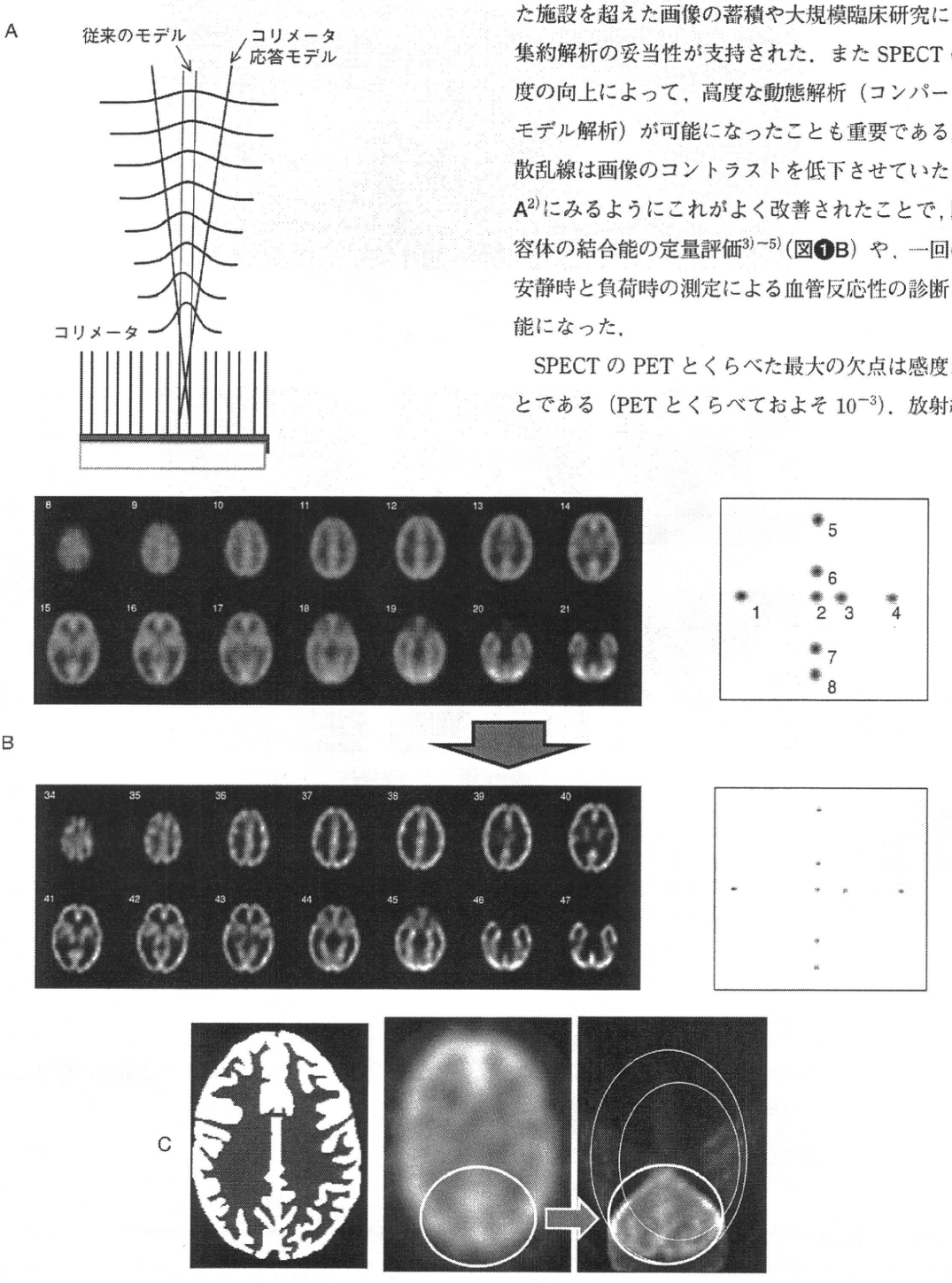
10^8にもなる LOR 信号を演算処理・保存するためには相当高速な電子回路を含む処理技術が必要である．また，3D PET 計測における散乱線を補正する普遍的な方法も確立されなければいけない．放射線分布と吸収減弱（μ）分布に物理公式（Klein-Nishina の式など）を適用する方法が提案されているが，視野外の放射能や散乱線発生の影響は意外に少ないことが当該研究グループ平野らによって明らかにされ，この方法の妥当性，また視野シールドの有効性が確認されたところである．いずれにせよ神経イメージングに向けた PET 計測技術は着実に進んでおり，さらに明瞭かつ高精度な画像の撮像が可能になる．

2 SPECT

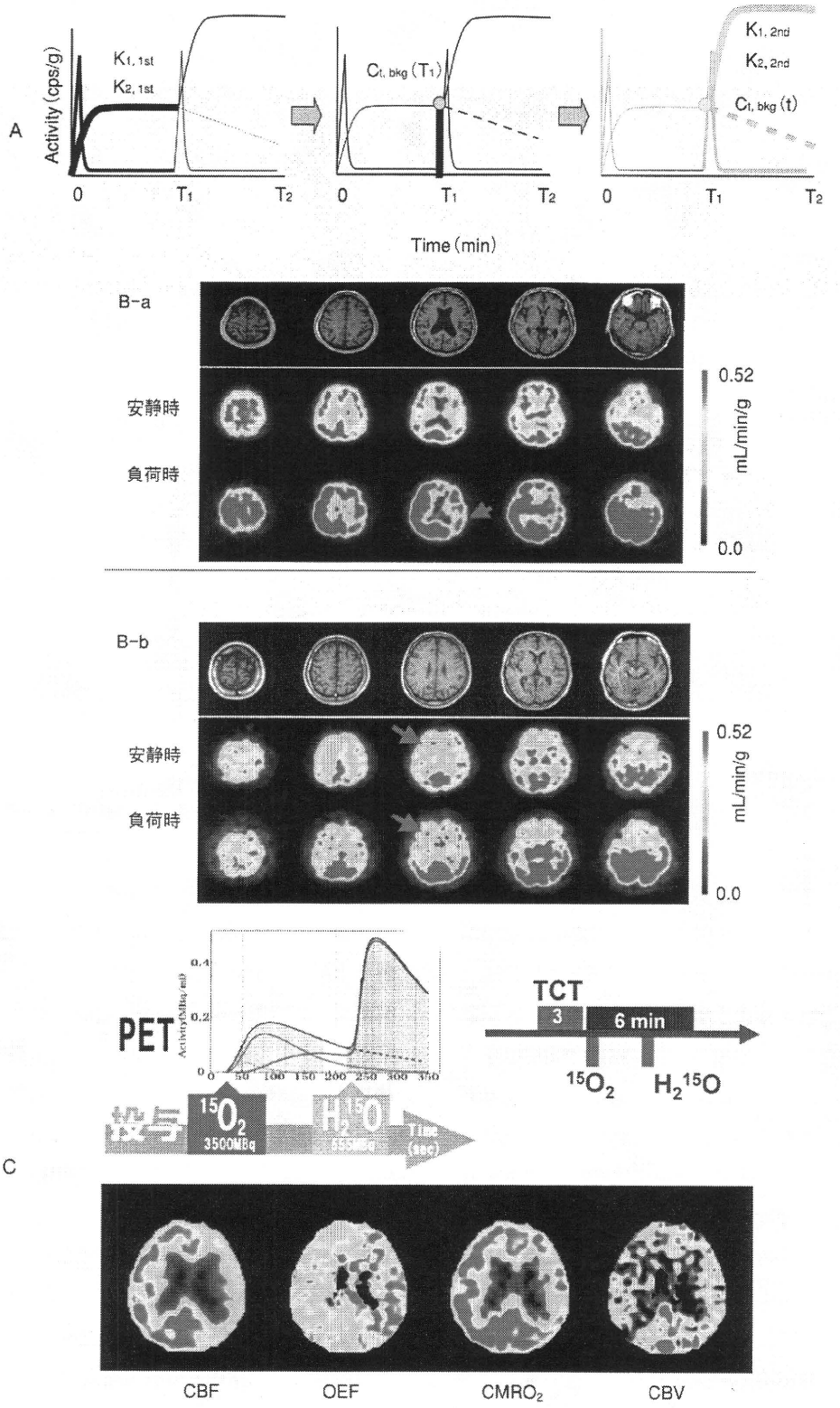
SPECT 技術の最近の重要な進展は，定量化と標準化に向けた活動であろう．SPECT 画像の定量精度の確保は困難とされてきたが，吸収と散乱線を比較的正確に補正する実用的なプログラムの整備によって，メーカーや機種を超えた再現性が得られるようになった．これは施設を

超えてデータが共有できる点においても，また既存の装置がそのまま利用できる点においても，PET に勝る重要な特長である．SPECT を使った多施設臨床研究は困難とされてきたが[1]，当該研究者らが中心となっておこなった施設を超えた画像の蓄積や大規模臨床研究において，集約解析の妥当性が支持された．また SPECT の定量精度の向上によって，高度な動態解析（コンパートメントモデル解析）が可能になったことも重要である．とくに散乱線は画像のコントラストを低下させていたが，図❶A[2]にみるようにこれがよく改善されたことで，脳神経受容体の結合能の定量評価[3)–5)]（図❶B）や，一回の撮像で安静時と負荷時の測定による血管反応性の診断などが可能になった．

SPECT の PET とくらべた最大の欠点は感度が低いことである（PET とくらべておよそ 10^{-3}）．放射線計測の

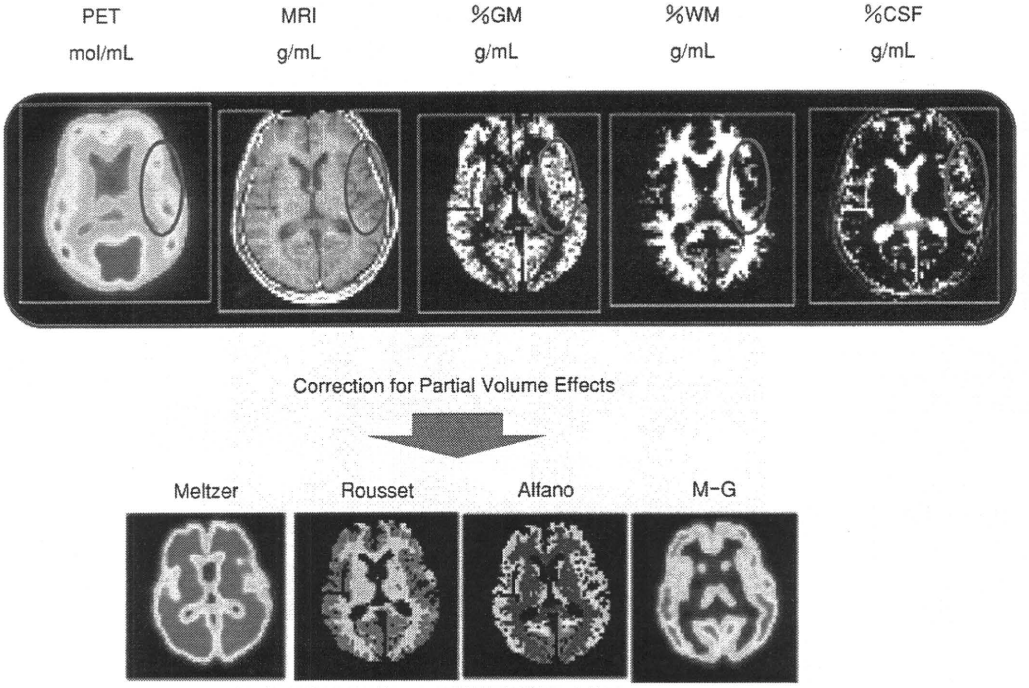


図❷ SPECT 画像再構成における空間解像度の補正
A：コリメータ開口にもとづく空間解像度劣化のモデル．B：コリメータ開口の補正を組み込むことで，空間解像度は 9.0 mm から 5.0 mm 以下に改善し，また統計ノイズも軽減された．今後臨床診断での実用化が望まれる．C：局所領域にピンホールコリメータの焦点を設定して得た 3D Hoffman ファントムの Tc-99m 画像．従来の画像撮像よりも局所において空間解像度の上昇が確認される．



図❸ 複数薬剤投与法の効果

A：複数トレーサ投与時の入力関数と脳内放射能濃度曲線を示す．バックグランド画像を推定し，さらに2回目投与後の脳内放射能濃度から機能画像を推定することが可能である．B：I-123 IMP の2回連続投与法にもとづく典型的な安静時とダイアモックス負荷後の局所脳血流量画像．症例 a では軽度血管狭窄を予測し，症例 b では高リスク血管狭窄を予測した．C：迅速^{15}O-ガス PET 検査への利用．従来1時間以上を要していた一連の検査が，10 分間以内のスキャンで可能になった．



図❹ MRI 形態画像の部分容積効果の補正への利用

一般におこなって感度の高いトレーサ計測技術ではあるが，精細な画像を得るにはやはり限界とされてきた．コリメータを工夫することで，原理的には限りなく高い空間解像度を得ることができるが，実際には空間解像度を犠牲にして現実的な感度を確保してきたのがこれまでの現況である．しかし近年になって，コリメータ開口による解像度劣化を補償するような立体的な画像再構成が試みられるようになり（**図❷A, B**），これは空間解像度の向上だけでなく統計ノイズの抑制の効果を有することが明らかになり注目されている．視野全体の計測データを局所の画像再構成に組み入れられることの効果であると理解できる．空間解像度と画質の両方が同時に改善できることの意義は，実際の臨床診断ではきわめて重要である．多くの応用領域に貢献することが期待される．

実験小動物の SPECT イメージングではピンホールコリメータを使って高い空間解像度が実現でき，また従来から問題とされた空間解像度の不均一さは撮像軌道の工夫[6]や，複数ホールコリメータの利用によってほぼ解決した．さらに，ヒトなど大きな対象においてもトランケーションによるアーチファクトを回避する理論が開発され，局所を高解像度撮像できることが示された（**図❷C**）．更なる技術整備によって実用化が待たれる．

3 動態解析の進歩

核医学の動態解析における課題の一つは，動的な機能変化の検出であろう．従来から，核医学イメージングでは 1 回の放射性薬投与に対してひとつの機能をみるに限られていたが，**図❸A** に示すように複数投与した動態解析において残存薬剤の影響を動態に組み込む理論が提案された[7][8]．また，PET や SPECT で撮像した画像は，検査中における平均ではなく過渡的な重みを有すること[9][10]を応用して，検査中の組織血流量やシナプス間隙の内因性神経伝達物質の濃度の時間変化の画像化が試みられている．また，脳賦活によるドーパミンリリース変化のタイミングをとらえる試みもなされ，この遅れがある種の疾患の本質であるとしている[11][12]．**図❸B** には SPECT 検査中に I-123 標識 iodoamphetamine（IMP）を 2 回投与し，安静時と血管拡張薬（ダイアモックス）投与による血管反応性の検査結果の例を示す．明らかに脳虚血の程度や脳梗塞発症のリスクを診断できるとして期待されている．また**図❸C** には，短時間の間に ^{15}O-標識酸素ガスと ^{15}O-標識水を連続投与し，従来 1 時間以上要していた検査が全体で 6〜9 分間の PET 撮像のみから局所脳血流量（CBF），局所脳酸素代謝量（$CMRO_2$），酸