Distribution of Cortical Benzodiazepine Receptor Binding in Right-Handed Healthy Humans: A Voxel-Based Statistical Analysis of Iodine 123 Iomazenil SPECT with Partial Volume Correction

BACKGROUND AND PURPOSE: CBR imaging is highly susceptible to a PVE produced by morphologic changes in the brain related to aging and brain laterality. We assessed the influence of PVE produced by regional age-related changes in gray matter volume on I-123 iomazenil SPECT and elucidated the age-related changes in human CBR binding by using PVE-corrected SPECT images.

MATERIALS AND METHODS: Nineteen right-handed healthy volunteers (range, 25–82 years; mean, 55 ± 21 years) underwent MR imaging and quantitative I-123 iomazenil SPECT imaging. The influence of age-related changes in rGMC on SPECT images before PVE correction was assessed. PVE correction of the SPECT images was performed by using an MR imaging–based method. Voxel-based linear regression analyses of the PVE-corrected SPECT images were performed by using SPM5.

RESULTS: The age-related reductions in rGMC and BP without PVE correction revealed a significant direct proportional correlation. Voxel-based statistical analysis with PVE correction showed no significant age-related changes in BP.

CONCLUSIONS: PVE correction was indispensable for the analysis of I-123 iomazenil SPECT images. PVE-corrected quantitative I-123 iomazenil SPECT images revealed no age-related changes in CBR binding in right-handed healthy humans.

ABBREVIATIONS: AAL = automated anatomic labeling; BP = binding potential; CBR = central benzodiazepine receptor; FWHM = full width at half maximum; I-123 = iodine 123; PSF = point-spread function; PVE = partial volume effect; rGMC = regional gray matter concentration; SPM = statistical parametric mapping; VBM = voxel-based morphometry

Materials and Methods

Subjects

Nineteen right-handed volunteers (10 men and 9 women; mean age, 58 ± 20 years; range, 25–82 years) were recruited for this study. The criterion for right-handedness was a laterality quotient of >75 according to the Edinburgh Handedness Inventory. None of the volunteers had a history of neurologic or psychiatric symptoms; furthermore, none of the volunteers exhibited abnormal T1-weighted or T2-weighted MR imaging findings. Each subject was asked to complete a cognitive function screening test by using the Japanese version of the Neurobehavioral Cognitive Status Examination Battery, Cognistat, on the day of the SPECT examination. Some of the elderly subjects were also asked to complete a memory function test (Wechsler Memory Scale-Revised). No abnormalities in cognitive function were observed in any of the subjects.

The study was approved by the ethics committee of Osaka University Hospital for Clinical Research.

SPECT

In each subject, 222 MBq of I-123 iomazenil was intravenously administered. SPECT images were acquired twice sequentially to quantify the receptor binding by using a simplified method. Early SPECT images were acquired immediately after injection, and delayed SPECT images were acquired 162 minutes after injection (the midscan time of the delayed scan was 180 minutes after injection). Venous blood samples were drawn from the arm contralateral to the injection site at 30 minutes postinjection. The SPECT scanning was performed by using a 4-headed gamma camera (Gamma View SPECT 2000H;...
Hitachi Medical, Tokyo, Japan) with a low-energy middle-resolution thin-section parallel-hole collimator. SPECT images were acquired every 22.5 seconds until 36 minutes, with 96 collections over 360°, and the data were recorded in a 64 × 64 matrix. The raw SPECT data were added to make an image and transferred to a nuclear medicine computer (HARP 3; Hitachi Medical). The projection data were prefiltered by using a Butterworth filter (cutoff frequency, 0.20 cycles/pixel; order, 10) and reconstructed into transaxial sections of 4.0-mm-thick images in planes parallel to the orbitomeatal line by using the filtered back-projection method. Attenuation correction was performed by using the Chang method, with an optimized effective attenuation coefficient of 0.12 cm⁻¹.

A quantitative analysis was performed by using the table look-up procedure, based on the 3-compartment 2-parameter model. We adopted a noninvasive version of this method by using a standardized arterial input function and a single venous blood sample, to minimize the invasiveness of the procedure. The measurement of the absolute 1-123 iomazenil BP in healthy humans is reasonably accurate by using this model, and the method has been validated in previous studies.

Briefly, parameters $K_1/k_2$ were fixed at 3.00, and $k_4$ was fixed at 0.26 throughout the study. The arterial input function was estimated by using the venous blood sample, as described above. On the basis of these fixed parameters and the arterial input function, 2 look-up tables were generated, in which $K_1$ and $k_3$ were uniquely determined. As previously described, the I-123 iomazenil BP can be calculated according to the following equation:

$$BP = \frac{1}{f_1} \frac{K_1 k_2}{k_3} k_4,$$

where $f_1$ is the free fraction of the parent compound in the plasma, which was fixed at 0.24 as determined in a previous study.

Fig 1. A, 1-123 iomazenil SPECT image. B, Automatic coregistration of the I-123 SPECT image with the MR image via smoothed gray matter maps. The maps are simultaneously reformatted to a matrix that is the same size as the referenced smoothed gray matter map. C, 3D MR image obtained before surgery. D, MR image segmented into Bayesian probability maps showing 3 tissue classes (gray matter, white matter, and CSF maps). E, The gray matter map convoluted with the PSF, which is assumed to be the same as the PSF of the SPECT scanner. The resultant image is subsequently referred to as the rGMC map. F, Smoothed gray matter map masked with a threshold set to 35% of the maximum voxel value. The coredistered I-123 SPECT image is divided by using the masked smoothed gray matter map on a voxel-by-voxel basis. G, Image (F) anatomically normalized by the spatial voxel value. H, Image (F) anatomically normalized in the same manner as in image (G).
MR Imaging

MR imaging was performed by using a Signa Excite 3T scanner (GE Healthcare, Milwaukee, Wisconsin). A 3D structural MR image was acquired for each subject by using a T1-weighted spoiled gradient-recalled echo sequence (TR/TE, 1.916/8.68 ms; flip angle, 18°; matrix size, 512 × 512; yield, 248 axial sections; section thickness, 1.4 mm; in-plane resolution, 0.43 × 0.43 mm) and T2-weighted 2D fast spin-echo sequences (axial plane; FOV, 250 mm; matrix, 512 × 512; section thickness, 5 mm; intersection gap, 1–1.5 mm; TE, 90–131 ms; TR, 4500–5000 ms).

Regional Gray Matter Concentration Maps

All the procedures were performed by using a personal computer (Dell Dimension 8300; Dell, Round Rock, Texas) running Microsoft Windows XP (Microsoft, Redmond, Washington).

The 3D T1-weighted MR image was resliced in the native space of each subject by using a voxel size of 1.0 × 1.0 × 1.0 mm. The results were first segmented into gray matter, white matter, and CSF and were then spatially normalized by using the unified model1 of SPM5 (Wellcome Department of Imaging Neuroscience: http://www.fil.ion.ucl.ac.uk/spm/) according to the optimized VBM protocol.3,12 In this study, however, we skipped the “modulation” step by multiplying the gray matter maps by the Jacobian determinants of the corresponding spatial normalization matrices, which corrects the local volumetric contraction or dilation that the images undergo during warping. These procedures generated both spatial normalization matrices and inverse spatial normalization matrices. The normalized map of the gray matter yielded in the above procedures was transformed into native space by using an inverse spatial normalization matrix. The resulting gray matter map in the native space was convoluted with a 3D Gaussian function with a FWHM of 12 mm.

PVE Correction

The PVE of the acquired SPECT images influences the estimated parameters. In a 3-compartmental model, the tissue activity $B(t)$ is given by the formula:

$$B(t) = \int_0^t C_a(s) f(t - s) ds,$$

where $C_a(t)$ is the arterial input function and $f(t)$ is the transfer function given by the formula:

$$f(t) = \frac{(k_1 + k_3 - \alpha_3)e^{-\alpha_3 t} - (k_1 + k_4 - \alpha_4)e^{-\alpha_4 t}}{\alpha_1 - \alpha_3},$$

where

$$\alpha_{1,2} = \frac{(k_2 + k_3 + k_4) \pm \sqrt{(k_2 + k_3 + k_4)^2 - 4k_2k_4}}{2}.$$

The PVE-uncorrected tissue activity observed at a certain voxel $B'(t)$ is approximately indicated as follows:

$$B'(t) = gB(t),$$

where $g$ is the rGMC value at the voxel. Because $g$ is a time-independent factor, according to the equations 2, 3, and 4, the parameter $K_1'$ corresponding to $B'(t)$ is given by the formula:

$$K_1' = gK_1,$$

whereas other parameters $K_i$ corresponding to $B'(t)$ are identical with $k_i$. According to equation 1, $BP'$ is given by the equation:

$$BP' = \frac{1}{f_1} \frac{K_1' k_3}{k_1} = g \frac{1}{f_1} \frac{K_1 k_3}{k_1} = gBP.$$
of the maximum rGMC, an empirically determined value) than that used in previous studies\(^2,13\) to minimize the error produced by the smoothing of the masked images before the voxel-based statistical estimation. These masked images based on the rGMC were then applied to the coregistered BP images. The masked BP images were then divided by using the rGMC map on a pixel-by-pixel basis. The threshold for masking, on the other hand, was set at 35% of the maximum rGMC (the same as the threshold value used in previous studies\(^2,13\)) when displaying the PVE-corrected BP images (Fig 1).

**Automated VOI Analysis**

Before the VOI analysis, each SPECT image was coregistered with the corresponding rGMC map and then spatially normalized by using the same normalization matrices as those generated in the above-mentioned normalization process for the rGMC map. Automated VOI analysis was performed for the normalized images by using an in-house tool based on normalized VOI templates. With this tool, voxels with a zero or negative value were automatically eliminated from the VOI to avoid selection of irrelevant voxels. In the present study, the

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Fig 3. Flow diagram showing the preprocessing steps for the VOI analysis and voxel-based statistical analysis protocols.

Fig 4. Age-related change in the rGMC extracted from MR imaging versus that from I-123 iomazenil BP uncorrected for PVE is indicated in the scatterplot graph (A). The data were evaluated by using a VOI analysis based on the AAL atlas, composed of 116 VOIs. For each VOI, the ratio of the group-averaged parameter for the young subjects to that for the elderly subjects is plotted. SPM analysis based on a multiple regression model for PVE-uncorrected SPECT images (B1) and for rGMC (B2) shows clusters of voxels with significant age-related decreases in the parameters.
AAL atlas was used as the template for the VOI analysis (Fig 2). Automated VOI analyses for the rGMC map and PVE-uncorrected BP maps were performed to elucidate the effect of PVE associated with age-related rGMC changes on the PVE-uncorrected SPECT BP images (Fig 3). The subjects were divided into 2 groups: the young group (40 years of age or younger) and the elderly group (older than 60 years). The correlations between the young-to-elderly ratio of the group-averaged rGMC and that of the group-averaged PVE-uncorrected BP were assessed for all 116 AAL regions.

Voxel-Based Statistical Analysis

Before the voxel-based statistical analysis, all the SPECT images were spatially normalized in the same way as mentioned above (Fig 3). All the normalized images prepared for the SPM analysis were smoothed by using a 12-mm FWHM isotropic Gaussian kernel. This step conditions the residuals to conform more closely to the Gaussian random field model underlying the statistical process used for adjusting the P values. After smoothing, the normalized binary mask images were also applied to the analysis as explicit mask images, to exclude irrelevant voxels corresponding to an rGMC of <35% of the maximum from the analysis. Global scaling was not applied to the voxel-based statistical analysis used in the present study.

The SPM statistical model used voxel-by-voxel multiple regression analyses to detect voxels showing a significant correlation between the rGMC and age (ie, VBM) or SPECT BP and age. In these models, age was included as a covariate of interest and sex was cast as a nuisance variable. The resultant set of voxel values constituted a statistical parametric map of the t statistic t-score map. The significance threshold was set at P < .05, with family-wise error correction for the peak height; no cluster size threshold was set.

Results

The VOI analysis of the rGMC and PVE-uncorrected BP images demonstrated a significant correlation between the age-related degree of reduction of the PVE-uncorrected BP and that of the rGMC, with the former being in direct proportion to the latter (r = 0.806, P < .001, Spearman rank correlation) (Fig 4A). The results of SPM analyses for the rGMC and PVE-uncorrected BP images by using voxel-based multiple regression analysis revealed clusters of voxels showing significant negative correlations with age in similar brain regions in both the analyses (Fig 4B1 - B2).

In a comparison of the averaged images between the young and elderly subjects, though the age-related decrease of the rGMC and PVE-uncorrected BP were similar (Fig 5A, -B), no age-related changes were found in the PVE-corrected BP (Fig 5C). The SPM multiple regression analysis for the PVE-corrected BP images showed no significant age-related changes of the BP, even when a liberal cutoff threshold (P < .1 with false discovery rate correction) was used.
Discussion

In the I-123 iomazenil BP images without PVE correction, age-related BP changes that were directly proportional to the age-related rGMC changes were observed. The degree of PVE on the SPECT images was associated with the rGMC, because I-123 iomazenil binding is limited to the gray matter. Therefore, the PVE-uncorrected BP images contained "apparent" variations caused by the PVE associated with the rGMC changes. An MR imaging-based correction for the PVE on I-123 iomazenil SPECT demonstrated the absence of any significant age-related changes in CBR binding.

A previous in vivo study on the rat brain did not reveal any age-related variations of CBR binding. Furthermore, a study of healthy humans by using PET, which has a much higher spatial resolution than SPECT, also showed no age-related reduction in CBR binding. According to the results of receptor autoradiography in human postmortem materials by using H-3 flunitrazepam, age had no influence on the receptor distribution or attenuation. Our findings in the present study support these previously reported findings and suggest that correction for PVE is essential for assessing CBR binding by using low-resolution imaging modalities such as SPECT. In a recent rat study by using [11C]flumazenil micro-PET, significant age-related decrease of the radioligand uptake in some brain regions was reported. On the other hand, a previous study showed age-related brain atrophy by noninvasive MR imaging in the same rats. Even with the high-resolution micro-PET system used in the study (FWHM, 2.0 mm), it seemed impossible to neglect the PVE associated with brain atrophy in the small rat brain.

The rationale for including only right-handed subjects in this study is that the distribution of the gray matter concentration between the 2 cerebral hemispheres has been reported to differ depending on the handedness, and the effect of handedness on the distribution of CBR binding is unclear. Further study by using PVE-corrected imaging of left-handed subjects is needed to clarify the handedness-related differences in the distribution of CBR binding.

In clinical settings, PVE-uncorrected I-123 iomazenil SPECT imaging might be useful when an age-matched normal database acquired with the same scanner is used for comparison. SPECT imaging might be useful when an age-matched normal database acquired with the same scanner is used for comparison. Thus, voxel-based statistical analysis with PVE correction and anatomical left-right asymmetry in young adult males.

Conclusions

The PVE in I-123 iomazenil SPECT images was found to show a directly proportional correlation with the regional age-related changes in the gray matter concentration. PVE correction was indispensable for the analysis of I-123 iomazenil SPECT images. PVE-corrected quantitative I-123 iomazenil SPECT imaging revealed no age-related changes in CBR binding in right-handed healthy humans.

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