Learning objectives

To evaluate age-related changes in the brain distribution of benzodiazepine receptors using I-123 iomazenil (IMZ).
Background

I-123 iomazenil (IMZ) is a radioiodinated ligand suitable for the assessment of central-type benzodiazepine (BZD) receptors by single photon emission computed tomography (SPECT) (Beer et al., 1990). IMZ binds to #-subunits of the gamma-aminobutyric acid receptor (GABA\(_A\) receptor) complex and is a BZD antagonist (Beer et al., 1990). GABA\(_A\) receptors are widely distributed as inhibitory receptors over all cortical areas. BZD binds to # and # -subunits of the GABA\(_A\) receptor, and binding of BZD-related ligands can enhance or decrease the electrophysiological effects of GABA by the modulating chloride ion flux (Cooper et al., 1996). The preserved density of GABA\(_A\) receptors is related to the presence of viable functional neurons, whereas the reduced density of these receptors is related to the loss of cortical synapses or neuronal cells. Flumazenil (FMZ) was developed as a BZD antagonist that can be labeled with C-11, and has been used for in vivo mapping of BZD receptors in clinical studies (Hunkeler et al., 1981; Pappata et al., 1988). Although evaluation of BZD receptors with FMZ is of great clinical interest, its use has been limited to institutions where positron emission tomography (PET) is available. IMZ was developed as an analogue of FMZ that could be labeled with I-123, and was intended for routine clinical use (Beer et al., 1990). The physical properties of I-123 are suitable for SPECT imaging; moreover, its high affinity to BZD receptors renders this ligand practical for clinical BZD receptor imaging (Venz et al., 1998). To date, several studies have investigated this ligand, suggesting its feasibility for in vivo BZD receptor mapping and its usefulness in the evaluation of various pathological conditions of the central nervous system.

Understanding the processes of brain maturation from infancy to adulthood is very important since these involve neural reorganization contributing to both normal variations and neurodevelopmental disorders, such as schizophrenia, the disease onset of which occurs in adolescence (Keshavan et al., 1994; Whitford et al., 2007). Thus far, several studies have suggested that human brain maturation is incomplete at birth and continues to progress until adulthood (Huttenlocher and de Courten, 1987; Purves, 1988; Huttenlocher and Dabholkar, 1997; Chugani et al., 2001; Sisk and Foster, 2004). In a study that assessed BZD receptors using FMZ and PET, the FMZ volume of distribution, a quantitative index of synaptic density, was analyzed in detail by cross-sectional analysis (Chugani et al., 2001).

In this context, we hypothesized that the distribution of IMZ may change with aging as brain maturation progresses. Although IMZ has been widely used to evaluate the distribution of BZD receptors, the age-related distribution of IMZ in humans has not been studied in detail. If the normal distribution of IMZ differs by age, these differences need to be characterized for IMZ SPECT in order to avoid incorrect diagnoses. In particular, it would be important to assemble an age-specific normal database in statistical image analysis. So far, IMZ SPECT imaging has been evaluated mainly by visual inspection or by semiquantitative approaches such as a ratio of radioactivities in regions of interest.
(ROIs) or the ROI washout rates from cross-sectional studies. Recently, some authors have reported that three-dimensional stereotactic surface projections (3D-SSP, Nihon Medipysics, Hyogo, Japan) could be useful for detecting subtle changes in tracer distribution in the brain (Minoshima et al. 1995; Imabayashi et al. 2004; Yasunori et al. 2005). In this study, we have analyzed IMZ SPECT images using statistical image analysis software (Minoshima et al. 1995) to identify age-related changes in regional IMZ brain distribution from infancy to adulthood.
Imaging findings OR Procedure details

Subjects

From 85 patients who underwent IMZ SPECT study in investigation for suspected epilepsy, we selected 29 subjects (M/F = 15/14; mean age ± SD, 18.0 ± 16.2 years; age range, 0 to 54 years) who did not show abnormal findings on IMZ SPECT and MRI, were selected for the study. A limitation of this study is that the subjects were not healthy volunteers but those suspected of having epilepsy; however, this selection bias was for ethical reasons. These subjects were classified into 4 groups by age; 1-5 years (n = 8, M/F = 5/3; mean age ± SD, 2.6 ± 1.4 years), 6-12 years (n = 7, M/F = 3/4; mean age ± SD, 7.9 ± 2.2 years), 13-30 years (n = 8, M/F = 5/3; mean age ± SD, 21.0 ± 5.1 years), and 31 years and older (n = 7, M/F = 2/5; mean age ± SD, 42.6 ± 6.2 years).

Data acquisition

At 3 h after the intravenous injection of 167 MBq of IMZ (Nihon Mediphysics, Hyogo, Japan), 60 projection data were acquired in a 128 × 128 matrix (pixel size: 1.7 × 1.7 mm) using an energy window of 10% centered at 160 keV, in a 360-degree step rotation mode with an acquisition time of 60 s each, by means of a triple- head digital gamma camera equipped with low- energy super high- resolution fan-beam collimators (GCA 9300A/DI; Toshiba Medical, Tokyo, Japan). The in-plane spatial resolution of this gamma camera was 7 mmFWHM. Image reconstruction was performed using a filtered back-projection method with a ramp filter after preprocessing with a Butterworth filter (cutoff frequency, 0.647cycle/cm; order, 8) to obtain transaxial SPECT images with 3.4 mmthickness.

Statistical Analysis

Statistical image analysis was performed with 3D-SSP and its related statistical analysis software package, which was developed by Minoshima et al (1995). One of the modules in this package that conducts an unpaired t-test between two non-corresponding groups (iSSP35_2tZ) was adopted to analyze the differences in IMZ distribution between the different age groups. The software first performed anatomical standardization and pixel count normalization of individual SPECT images. Then, brain surface projection was performed to create brain surface images and the associated pixel data files. The brain surface pixel data were used to perform unpaired t-tests and correlation analyses on a pixel-by-pixel basis to create Z-score images. The Z-score was calculated by transforming the t statistic to the normal distribution using a probability integral transformation. The results were shown as two Z-score images for correlation analyses (positive and negative correlations) and unpaired t-tests (increase and decrease). From the results of the correlation analyses, the anatomical brain regions showing significant correlations with age were determined using the Talairach Daemon Client software.
(Version 1.1; Research Imaging Center, University of Texas Health Science Center, San Antonio, TX) (Lancaster et al., 2000). The significance level was set to $P < 0.05$ with Bonferroni’s multiple comparison correction. Further, $P < 0.005$ without correction was also used. Since the number of analyzed pixels on the brain surface projection was 15964, the corresponding $Z$-scores for $P < 0.05$ with the multiple comparison correction and $P < 0.005$ without correction were $Z = 4.52$ and $Z = 2.58$, respectively.

RESULTS

We successfully obtained and transformed 29 SPECT scans to brain surface projection images by 3D-SSP. Using these brain surface projection images, mean and SD images for each age group were generated after pixel count normalization (Fig. 1 on page 7). In the mean images thus generated, we noted that the IMZ distribution around the central sulci and cerebellum decreased with increasing age. In contrast, the IMZ distribution in the frontal, temporal, and parietal lobes increased with age. The results of the unpaired $t$-test (iSSP35_2tZ) confirmed our observations from the mean images. A more significant decrease in IMZ distribution was observed around the central sulci, occipital lobes, and cerebellum with increasing age, using the youngest age group (1-5 years) as a control group (Fig. 2 on page 7). On the other hand, a more significant increase in IMZ distribution was observed in the frontal, temporal, and parietal lobes with increasing age (Fig. 3 on page 8). In Fig. 3 and 4, the cut-off $Z$-score value was set to $Z = 1.64$ ($P < 0.05$ with multiple comparison correction). Although some pixels in the brain stem showed a high $Z$-score in the decreased $Z$-score map (Fig. 2 on page 7), this could be spurious since the IMZ distribution in the brain stem was very sparse as observed in the mean IMZ distribution images (Fig. 1 on page 7).

Pixel-by-pixel correlation analysis between IMZ distribution and age showed similar results to those observed in the results of unpaired $t$-test. Results are expressed in two different $Z$-score maps for positive and negative correlations (Fig. 4 on page 9). Corresponding brain regions (the nearest gray matter) to the pixels with high $Z$-scores ($Z > 2.58; P < 0.005$ without multiple comparison correction) was determined by the Talairach Daemon software. The results for negative and positive correlations are summarized in Tables 1 and 2. The brain region that showed negative correlations between IMZ distribution and age mainly included the precentral gyri related to motor activity. In contrast, positive correlations were noted in the broader areas of the frontal, temporal, and parietal lobes. These regions are related to higher brain functions, such as cognitive functions and judgment.
**Fig. 1:** Mean images of the 4 age groups. IMZ distributions around the central sulci and cerebellum decreased with increasing age. In contrast, those in the frontal, temporal, and parietal lobes increased with age.

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Fig. 2: Decreased Z-score maps (1-5 y group vs. other age groups). A significant decrease in IMZ distribution was observed around the central sulci, occipital lobes, and cerebellum with increasing age.

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Fig. 3: Increased Z-score maps (1-5 y group vs. other age groups). A significant increase in IMZ distribution was observed in the frontal, temporal, and parietal lobes with increasing age.

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Fig. 4: Correlation Z-score maps (A: negative, B: positive). The brain regions showing negative correlations between IMZ distribution and age were in the pericentral regions, occipital lobes, and cerebellum. On the other hand, positive correlations were noted in the broader areas of the frontal, temporal, and parietal lobes.
Table 1: Brain regions that showed significant negative correlation with age (P < 0.005 without multiple comparison correction)

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Table 2: Brain regions that showed significant positive correlation with age (P < 0.005 without multiple comparison correction)

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Conclusion

In conclusion, our findings confirmed age-related changes in the brain distribution of IMZ. IMZ distribution in the brain appears to change sequentially with aging as maturation progresses. Our results may contribute to understanding regional differences in GABA_A receptor distribution as maturation occurs. Our study illustrates the powerful approach that 3D-SSP provides in the study of human brain development. We consider that in the future, it might be important to assemble an age-specific normal database to contribute precise diagnoses based on IMZ SPECT imaging.
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References


