Selective neuronal damage and Wisconsin Card Sorting Test performance in atherosclerotic occlusive disease of the major cerebral artery

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ABSTRACT

Background In atherosclerotic internal carotid artery (ICA) or middle cerebral artery (MCA) disease, selective neuronal damage can be detected as a decrease in central benzodiazepine receptors (BZRs) in the normal-appearing cerebral cortex. This study aimed to determine whether a decrease in the BZRs in the non-infarcted cerebral cortex is associated with poor performance on the Wisconsin Card Sorting Test (WCST), which assesses executive functions.

Methods The authors measured the BZRs using positron emission tomography and ^{11}C-flumazenil in 60 non-disabled patients with unilateral atherosclerotic ICA or MCA disease and no cortical infarction. Using three-dimensional stereotactic surface projections, the abnormally decreased BZR index (extent (%) of pixels with Z score >2 compared with controls×average Z score in those pixels) in the cerebral cortex of the anterior cerebral artery (ACA) or MCA territory was calculated and found to be correlated with the patient's score on the WCST.

Results On the basis of the WCST results, 39 patients were considered abnormal (low categories achieved) for their age. The BZR index of the ACA territory in the hemisphere affected by arterial disease was significantly higher in abnormal patients than in normal patients. The BZR index of the MCA territory differed significantly between the two groups when patients with left arterial disease (n=28) were analysed separately.

Conclusions In atherosclerotic ICA or MCA disease, selective neuronal damage that is manifested as a decrease in BZRs in the non-infarcted cerebral cortex may contribute to the development of executive dysfunction.

INTRODUCTION

Imaging of the central benzodiazepine receptors (BZRs), which are expressed on most cortical neurons, has made it possible to visualise in vivo the neuronal alterations induced by ischaemia in humans.[1–6] Previous studies have shown that in atherosclerotic internal carotid artery (ICA) or middle cerebral artery (MCA) disease, haemodynamic cerebral ischaemia induces selective neuronal damage manifested as a decrease in BZRs in the non-infarcted cerebral cortex.[7–9] In patients with chronic haemodynamic compromise, the perfusion may fall below the penumbra threshold for a matter of minutes, causing selective neuronal damage without any infarction visible on a MRI scan.[7–9] However, the impact of this type of damage on clinical outcomes is unclear. In patients with atherosclerotic ICA or MCA disease, a few patients with reduced BZRs in the cortex overlying subcortical infarcts have been reported to show aphasia,[2 10] and a decrease in cortical BZRs has been shown to be correlated with atrophy of the corpus callosum that is associated with global cognitive impairment.[11 12] However, the relationship between a decrease in BZRs and the neurological symptoms has not been systematically investigated.

Selective neuronal damage may contribute to the development of subtle poststroke cognitive impairment, depending on the degree of neuronal damage.[13 14] Executive dysfunction may be an early sign of vascular cognitive impairment and may have considerable impact on the functional outcomes of patients with stroke.[15 16] The Wisconsin Card Sorting Test (WCST) is a widely used clinical test for assessing executive functions.[17] The goal of this study was to determine whether a decrease in BZRs in the non-infarcted cerebral cortex is associated with poor performance on the WCST in patients with atherosclerotic ICA or MCA disease.

METHODS

Patients

We studied 60 consecutive right-handed patients with unilateral atherosclerotic occlusion or stenosis of the ICA or MCA (table 1). They were referred to our positron emission tomography (PET) unit during 16 months for evaluation of the haemodynamic effects of ICA or MCA disease as a part of a comprehensive clinical evaluation to determine the necessity of vascular reconstruction surgery (carotid endarterectomy, carotid stenting, or superficial temporal artery to MCA anastomosis). Inclusion criteria were as follows: (1) occlusion or stenosis of the ICA (>60% diameter reduction according to the NASCET criteria) or MCA (>50% diameter reduction) as documented by conventional or magnetic resonance angiography (MRA);[18 19] (2) ability to independently carry out daily life activities (score on modified Rankin scale <3); and (3) for patients with symptoms, history of transient ischaemic attack (TIA) or completed stroke involving the relevant ICA or MCA territory. TIA was defined as the development of focal symptoms of presumed ischaemic cerebrovascular origin lasting <24 h. The exclusion criteria were (1) cortical infarction detectable on routine MRI images (T1-weighted, T2-weighted or fluid-attenuated inversion recovery (FLAIR) images); (2) history

of TIA or stroke in regions other than the relevant ICA or MCA territory; (3) history of vascular reconstruction surgery; (4) contralateral ICA or MCA stenosis (>50%); (5) unilateral arterial disease with extensive white-matter lesions in both hemispheres; (6) history of consumption of BZR agonists or alcohol abuse; or (7) presence of potential sources of cardiogenic embolism, including recent myocardial infarction (<3 weeks previously), known atrial fibrillation, mitral stenosis, presence of a prosthetic mitral valve, dilated cardiomyopathy, sick sinus syndrome or acute bacterial endocarditis.

In the 36 patients with symptoms, the interval between the last ischaemic event and the PET evaluation ranged from 0.5 to 41 (mean (SD), 8 (10)) months for the 14 TIA patients and from 0.7 to 92 (17 (25)) months for the 22 patients with stroke. In the 24 asymptomatic patients, arterial disease was suspected because of the findings of MRA or echoangiography performed for dizziness, vertigo or loss of consciousness, or as part of screening for cerebral arterial disease concomitant with coronary arterial disease.

To obtain a normal control database for BZR imaging, we studied 10 healthy control subjects (aged 57 (7) years, including seven men), who had no previous history of medical or psychiatric disorders and no history of consumption of BZR agonists or alcohol abuse. The ethics committee of our centre approved the study protocol, and written informed consent was obtained from both patients and control individuals.

Wisconsin Card Sorting Test

The WCST is one of the most frequently used neuropsychological tests and is the most sensitive to frontal-lobe dysfunction.[17] In this study, we used the Keio–Fukuoka–Shimane version of the WCST, specifically, the Keio version of the newly modified WCST that can be performed on a personal computer (PC).[20 21] In this test, four stimulus cards were shown in the upper row of the PC test screen, and 48 response cards were shown in order in the lower row. By trial and error, the participants determined which stimulus card matched each response card, based on one of the three categories of colour, form and number selected by the computer. Simultaneously, the patients were requested to state which category they selected to evaluate impaired verbal regulation. The computer responded to the answer by voice with 'correct' or 'wrong.' Once the subject learnt to sort by one rule, after six consecutive correct responses, the initial sorting principle was changed without warning, shifting to a new category. The test continued until all 48 cards were used. The calculated indices included categories achieved, total errors, perseverative errors as defined by Nelson, non-perseverative errors and difficulty maintaining sets. Perseverative errors designated exact repetitions of the immediately preceding incorrect responses, whereas non-perseverative errors included all errors other than perseverative errors. Difficulty maintaining sets designated incorrect responses after two or more consecutive correct responses. For the categories-achieved index, a value of 3 or more for subjects aged ≥65 years old and a value of 4 or more for subjects aged ≤64 years old was determined to be normal.[21]

Table 1 Patient characteristics

Characteristics	
No of patients (n)	60
Age (mean±SD, years)	64±9
Sex, male/female (n)	45/15
Diagnosis (n)	
Asymptomatic	24
Transient ischaemic attack	14
Stroke	22
Subcortical infarction (n)	45
Frontal infarction (n)	31
ICA, stenosis (L/R) (n)	23 (9/14)
ICA, occlusion (L/R) (n)	20 (12/8)
MCA, stenosis (L/R) (n)	5 (2/3)
MCA, occlusion (L/R) (n)	12 (5/7)
Conventional angiography (n)	44
Wisconsin Card Sorting Test parameters (n)	
Categories achieved	2.4±1.8
Total errors	24.2±8.8
Perseverative errors (Nelson)	8.4±7.7
Non-perseverative errors	15.7±5.2
Difficulty maintaining sets	2.1±2.0

ICA, internal carotid artery; L/R, left/right; MCA, middle cerebral artery.

Positron emission tomography measurements

We performed PET scans using a whole-body Advance scanner (General Electric Medical System, Milwaukee, Wisconsin), which permits simultaneous acquisition of 35 image slices with interslice spacing of 4.25 mm.[22] A transmission scan was performed using $^{68}Ge/^{68}Ga$ for attenuation correction in each subject before the tracer administration. In reconstruction of PET data using filtered back projection, images were blurred to 6.0 mm full width at half maximum in the transaxial direction using a Hanning filter.

First, a series of ^{15}O-gas studies was performed.[22] $C^{15}O_2$ and $^{15}O_2$ were continuously inhaled through a mask. The one scan time was 5 min. Bolus inhalation of $C^{15}O$ with scanning for 3 min was used to measure the cerebral blood volume (CBV). Arterial blood was sampled two or three times during each procedure of the ^{15}O-gas study. After the ^{15}O-gas study, a ^{11}C-flumazenil (FMZ) study was performed.[7 23] ^{11}C-FMZ was synthesised by ^{11}C-methylation of demethylated-FMZ (Hoffmann-La Roche, Basel, Switzerland). Specific activities at the time of injection ranged from 16.2 to 20.2 (mean 17.8) GBq/μmol. After slow intravenous injection of 370–555 MBq of ^{11}C-FMZ into the right antecubital vein using an automatic injector over 60 s, a 50 min dynamic PET scan was started at the time of tracer administration with frame durations of 10 s × 6, 15 s × 8, 30 s × 4, 60 s × 5, 5 min × 4 and 10 min × 2.

We calculated the cerebral blood flow (CBF), cerebral metabolic rate of oxygen ($CMRO_2$) and oxygen extraction fraction (OEF) using the steady-state method.[24] The $CMRO_2$ and OEF were corrected on the basis of the CBV.[25] The binding potential (BP) of ^{11}C-FMZ was calculated using dynamic data and Logan graphical analysis with reference tissue, in which the pons was used as a reference region.[23 26] We selected two tomographic planes corresponding to the pons on the average tissue activity image obtained from the early phase of dynamic PET data with time frames of 1–3 min. We manually placed on these two planes an irregular region of interest (ROI) which included almost all of the pixels that had activity in the range from the maximum pixel value to 50% of the maximum value. This method was validated using the data on normal volunteers in comparison with identification of the pons on a coregistered MRI,[23] although it is ideal to set the ROIs on the coregistered MRI images in each patient. These ROIs were transferred to the dynamic PET data for the creation of parametric images of FMZ BP.

Magnetic resonance imaging

We performed MRI using a Signa unit (General Electric, Milwaukee, Wisconsin) operated at a field strength of 1.5 T. The imaging protocol consisted of a T2-weighted spin echo (repetition time/echo time ((TR/TE)=3000/88.8 ms), T1-weighted spin echo (TR/TE=550/11.2 ms) and FLAIR (TR/TE=8002/158 ms, inversion time=2000 ms) imaging series. The slice thickness was 5 mm, and the intersection gap was 2.5 mm.

Cerebral ischaemic lesions were identified as hyperintense lesions on T2-weighted MR images. FLAIR images were used to distinguish infarcts from dilated perivascular spaces. On the slice with the largest area of ischaemic lesions, the largest diameter of a lesion (A) and the largest diameter perpendicular to A (B) were measured, and A×B (mm^2) was calculated as an index of the size of the subcortical ischaemic lesion. The total quantity of subcortical ischaemic lesions was calculated by summing the indices for all lesions in the hemisphere affected by arterial disease. A single investigator who was blinded to the clinical status of the patients, including other imaging data, reviewed all scans.

Data analysis

A three-dimensional stereotactic surface projection (3D-SSP) technique was used to analyse FMZ BP, CBF and $CMRO_2$ parametric data.[27] Analysis by 3D-SSP anatomically normalises the individual PET data to the standard brain and compares the regional voxel data between patients and controls. This procedure was performed using the interface software iSSP (version 3.5; Nihon Medi-Physics Corporation, Nishinomiya, Japan). In brief, stereotactic reorientation of each brain into a standard shape is conducted using non-linear warping with the anterior commissure–posterior commissure line as the anatomic line of reference. The data are extracted by projecting the cortical activity onto the brain surface. In the standard stereotactic system, pixels located on the outer and medial surfaces of both hemispheres are predetermined along with 3D vectors perpendicular to the surface at each pixel. For each predetermined surface pixel, the algorithm searches for the highest pixel value in a direction inward along the vector to a 6-pixel depth into the cortex in an individual's anatomically standardised PET image set and assigns the maximum value to the surface pixel. Therefore, each brain was stereotactically transformed into a standard surface image format, which enabled us to compare the resultant cortical projections between the patients and controls. This 3D-SSP surface projection technique minimises the effects of cortical atrophy. For analysis of FMZ BP data, to negate the effect of fluctuations in whole-brain values and to extract changes caused by ICA or MCA disease, the pixel values of an individual's image set were normalised to the mean cerebellar value before the analysis. To quantify the decrease in FMZ BP, pixel-by-pixel Z scores were used, and Z scores ((mean normalised pixel value for controls−normalised pixel value for each patient)/SD for controls) were calculated for each surface pixel. A positive Z score relative to the controls represents a reduced FMZ BP in the patients. We considered Z scores >2 to be abnormal. To quantitatively assess the degree of FMZ BP reduction in each patient, the abnormally decreased BZR index (extent (%) of pixels with Z scores >2 compared with controls × average Z score in those pixels) in the cerebral cortex of the MCA, anterior cerebral artery (ACA) or posterior cerebral artery (PCA) territory was calculated using the stereotactic extraction estimation (SEE) method.[28] According to the Talairach Daemon, anatomical information was segmented into the gyrus level. Because vascular territories vary among individuals, we arbitrarily determined the MCA, ACA or PCA territories according to the atlas prepared by Kretschmann and Weinrich.[29] The MCA territory included the middle and inferior frontal gyri; precentral gyrus; superior and inferior parietal gyri; angular gyrus; postcentral gyrus; supramarginal gyrus; superior, middle, inferior and transverse temporal gyri; and superior and middle occipital gyri. The ACA territory included the superior and medial frontal gyri, orbital gyrus, rectal gyrus, paracentral lobule, subcallosal gyrus, orbital gyrus, precuneus, and cingulate and anterior cingulate gyri. The PCA territory included the inferior occipital gyrus, cuneus, fusiform gyrus, lingual gyrus, parahippocampal gyrus, posterior cingulated gyrus and uncus. For the analysis of CBF and $CMRO_2$ data, absolute values for each territory were calculated using the 3DSSP and SEE methods. The regional value of OEF was recalculated using the regional values of CBF, $CMRO_2$ and the arterial oxygen content.

Statistical analysis

Hemispheric differences in PET parameters in the three arterial territories were tested using a paired t test; significance was established at $p<0.05$. We compared the values of PET or clinical variables between WCST-abnormal (low categories achieved for their age) and normal groups using the Mann–Whitney U test or Fisher exact test, as appropriate; significance was established at $p<0.05$. Correlations between the total number of errors in the WCST and the BZR index for the subregions in the MCA or ACA territory of the affected hemisphere were analysed using multiple regression analysis after controlling for the effect of the patient's age; significance was established at $p<0.005$.

RESULTS

The BZR index, CBF, $CMRO_2$ and OEF of the hemisphere affected by arterial disease were significantly different from those of the non-affected hemisphere for the MCA and ACA territories (table 2), whereas hemispheric differences in the values of the BZR index, CBF, $CMRO_2$ and OEF were not significant for the PCA territory. On the basis of these results,

Table 2 Positron emission tomography results for hemispheres affected or unaffected by arterial disease

	Affected	Non-affected
Benzodiazepine receptor index (%)		
MCA	30.7±30.6*	13.0±12.3
ACA	16.0±16.5*	12.3±13.2
PCA	13.4±12.4	11.1±10.6
Cerebral blood flow (ml/100 g/min)		
MCA	43.4±8.1*	46.3±8.4
ACA	45.9±9.4*	47.0±9.4
PCA	48.4±9.4	49.0±9.4
Cerebral metabolic rate of oxygen (ml/100 g/min)		
MCA	3.65±0.41*	3.79±0.41
ACA	3.75±0.48*	3.81±0.52
PCA	4.08±0.46	4.13±0.43
Oxygen extraction fraction (%)		
MCA	49.6±6.6*	48.2±5.9
ACA	48.6±6.9*	47.9±6.8
PCA	48.7±5.9	48.8±5.8

Values are mean±SD.
Comparative values for CBF, $CMRO_2$ and OEF in the MCA, ACA and PCA distributions in normal volunteers were 52.5±6.0, 50.9±6.3, 60.3±7.1, 4.07±0.42, 3.80±0.40, 4.70±0.64, 44.8±3.4, 43.2±3.0 and 44.9±3.6, respectively.
*$p<0.05$, paired t test.
ACA, anterior cerebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery.

we selected the parameters of the affected hemisphere for the MCA and ACA territories for the analyses described below. The value of the BZR index was significantly correlated with the value of the OEF in the MCA (Spearman, $\rho=0.32$, $p<0.05$) or ACA ($\rho=0.29$, $p<0.05$) territory as well as with the value of the CBF or $CMRO_2$ in the MCA ($\rho=-0.36$, $p<0.01$ or $\rho=-0.51$, $p<0.001$, respectively) or ACA ($\rho=-0.38$, $p<0.005$ or $\rho=-0.28$, $p<0.05$) territory. Comparing patients with ICA and MCA disease, the BZR index in the MCA territory tended to be high in patients with MCA disease (ICA/MCA, mean (SD), 26.0 (22.9)/42.5 (43.2)), and the BZR index in the ACA territory tended to be high in patients with ICA disease (ICA/MCA, 17.7 (17.8)/11.9 (12.3)), but these differences were not statistically significant (Mann–Whitney U test).

For the categories-achieved index of the WCST, 39 patients were considered abnormal (low categories achieved) for their age. When we compared the values of the BZR index for the MCA and ACA territories between the abnormal and normal groups, the BZR index for the ACA territory was significantly increased in the abnormal group (figure 1), whereas the BZR index for the MCA territory tended to be increased in the abnormal group; these values were not statistically significant (table 3). The patient age, patient sex, side of arterial disease, incidence of patients with symptoms, incidence or size of subcortical infarctions, incidence of frontal infarctions, and the CBF and $CMRO_2$ for the MCA or ACA territories were not significantly different between the abnormal and normal groups. When we separately analysed the data in patients with left arterial diseases, the BZR indices for both the ACA and MCA territories were significantly increased in the abnormal group, as compared with the normal group (table 4). A separate analysis of the data in patients with ICA or MCA disease (table 5) or in symptomatic or asymptomatic patients (table 6) yielded results similar to those of the overall analysis, although the significant difference in the BZR index for the ACA territory disappeared in patients with ICA disease and in asymptomatic patients.

The total number of errors was significantly correlated with patient age (linear regression analysis, $r=0.39$, $p=0.0016$), but it was not significantly correlated with the quantity of subcortical ischaemic lesions ($r=0.07$, $p=0.59$). Among the BZR indices for the subregions of the ACA territory, those of the anterior cingulate gyrus ($p=0.0008$) (figure 2) and the medial frontal gyrus ($p=0.0012$) were significantly and positively correlated with the total number of errors after controlling for the effect of age by using multiple regression analysis. In patients with left arterial diseases, among the BZR indices for the subregions of the MCA territory, the BZR index of the middle frontal gyrus ($p=0.0015$) was significantly and positively correlated with the total number of errors after controlling for the effect of age in addition to those of the anterior cingulate gyrus ($p=0.0005$) and the medial frontal gyrus ($p=0.0001$).

Table 3 Characteristics of abnormal patients (low categories achieved on Wisconsin Card Sorting Test (WCST)) and normal patients

	Abnormal	Normal
No of patients (n)	39	21
Benzodiazepine receptor index (%)		
MCA territory (affected)	33.8±31.4	24.9±28.7
ACA territory	19.1±16.9*	10.4±14.6
Cerebral blood flow (ml/100 g/min)		
MCA territory	42.6±7.7	45.0±8.8
ACA territory	45.2±8.5	47.1±10.4
Cerebral metabolic rate of oxygen (ml/100 g/min)		
MCA territory	3.58±0.38	3.79±0.44
ACA territory	3.70±0.40	3.85±0.60
Age (years)	65±8	61±10
Sex, male/female (n)	28/11	17/4
Arterial disease, internal carotid artery/MCA (n)	28/11	15/6
Side of disease, left/right (n)	19/20	9/12
Symptomatic (n)	23	13
Subcortical infarction (n)	31	14
Size (mm^2)	137±194	142±208
Frontal infarction (n)	21	10
Wisconsin Card Sorting Test parameters (n)		
Categories achieved	1.4±1.0*	4.1±1.0
Total errors	28.6±7.3*	15.9±4.2
Perseverative errors (Nelson)	11.1±8.2*	3.5±3.0

Values are mean±SD.
*$p<0.05$, Mann–Whitney U test.
ACA, anterior cerebral artery; MCA, middle cerebral artery.

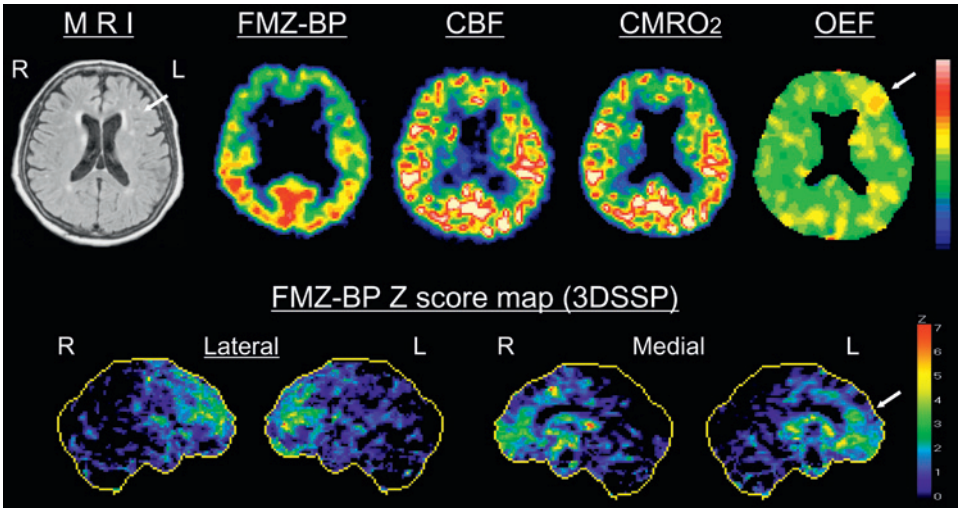

Figure 1 Examples of positron emission tomography images for a patient with left (L) internal carotid artery occlusion. This patient presented with transient ischaemic attacks, and the total number of errors on the Wisconsin Card Sorting Test was 48 (no correct responses). Angiography showed collateral pathways through leptomeningeal anastomosis from the left posterior cerebral artery and hypoplasia of the A1 segment of the right (R) anterior cerebral artery. Upper row: a decrease in the flumazenil-binding potential (FMZ-BP), cerebral blood flow (CBF) and cerebral metabolic rate of oxygen ($CMRO_2$) was observed in the bilateral anterior frontal regions. An increase in the oxygen extraction fraction (OEF) was observed in the left frontal region where small subcortical ischemic lesions were found on the corresponding MRI. Lower row: Z score maps from a three-dimensional stereotactic surface-projection (3DSSP) analysis showing a decrease in the FMZ-BP in the bilateral medial and lateral anterior frontal regions. The benzodiazepine receptor (BZR) index was 61.5 for the left anterior cerebral artery distribution.

Table 4 Characteristics of patients with left and right arterial disease

	Left		Right	
	Abnormal	Normal	Abnormal	Normal
No of patients (n)	19	9	20	12
Benzodiazepine receptor index (%)				
MCA territory (affected)	17.5±12.7*	8.9±12.7	49.3±36.2	37.0±31.9
ACA territory	15.7±16.0*	3.7±5.5	22.2±17.6	15.4±17.4
Cerebral blood flow (ml/100 g/min)				
MCA territory	44.3±7.5	47.4±6.9	40.8±7.8	43.1±10.1
ACA territory	46.9±9.2	50.1±5.8	43.4±7.5	44.7±12.8
Cerebral metabolic rate of oxygen (ml/100 g/min)				
MCA territory	3.74±0.35	3.95±0.40	3.41±0.35	3.67±0.46
ACA territory	3.82±0.40	3.96±0.55	3.56±0.37	3.78±0.66
Age (years)	63±8	58±10	67±9	64±9
Sex, male/female (n)	12/7	7/2	16/4	10/2
Arterial disease, internal carotid artery/MCA (n)	14/5	7/2	14/6	8/4
Symptomatic (n)	11/8	6/3	12/8	7/5
Subcortical infarction (n)	15	6	16	8
Size (mm^2)	97±98	155±241	176±252	132±191
Frontal infarction (n)	11	5	10	5
Wisconsin Card Sorting Test parameters (n)				
Categories achieved	1.2±1.1*	4.7±1.0	1.5±0.9*	4.2±1.0
Total errors	28.5±7.3*	15.6±4.0	28.7±7.5*	16.1±4.5
Perseverative errors (Nelson)	11.0±9.9*	3.0±2.2	11.3±6.4*	3.9±3.5

Values are mean±SD.
*$p<0.05$, Mann–Whitney U test.
Abnormal, low categories achieved on the Wisconsin Card Sorting Test; ACA, anterior cerebral artery; MCA, middle cerebral artery.

Table 5 Characteristics of patients with internal carotid artery (ICA) and middle cerebral artery (MCA) disease

	ICA		MCA	
	Abnormal	Normal	Abnormal	Normal
No of patients (n)	28	15	11	6
Benzodiazepine receptor index (%)				
MCA territory (affected)	24.4±24.6	19.7±18.4	44.9±43.9	38.1±45.5
ACA territory	20.2±18.3	12.9±16.4	16.1±13.0*	4.2±6.2
Cerebral blood flow (ml/100 g/min)				
MCA territory	42.8±6.7	44.9±7.3	42.0±10.4	45.3±12.6
ACA territory	44.4±6.7	45.8±7.3	47.2±12.2	50.3±15.9
Cerebral metabolic rate of oxygen (ml/100 g/min)				
MCA territory	3.53±0.32	3.81±0.48	3.72±0.51	3.73±0.35
ACA territory	3.60±0.31	3.82±0.61	3.95±0.51	3.94±0.66
Age (years)	68±7	63±11	58±6	58±6
Sex, male/female (n)	22/6	12/3	6/5	5/1
Side of disease, left/right (n)	14/14	7/8	5/6	2/4
Symptomatic (n)	15/13	8/7	8/3	5/1
Subcortical infarction (n)	21	10	10	4
Size (mm^2)	101±140	155±238	231±278	110±115
Frontal infarction (n)	13	6	8	4
Wisconsin Card Sorting Test parameters (n)				
Categories achieved	1.2±1.0*	4.2±1.1	1.7±1.1*	5.0±0.6
Total errors	30.6±6.8*	16.8±4.0	23.6±6.3*	13.8±4.3
Perseverative errors (Nelson)	12.7±8.5*	4.2±3.1	7.0±5.7*	1.8±1.9

Values are mean±SD.
*$p<0.05$, Mann–Whitney U test.
Abnormal, low categories achieved on the Wisconsin Card Sorting Test; ACA, anterior cerebral artery; MCA, middle cerebral artery.

DISCUSSION

This study showed that selective neuronal damage manifested as a decrease in the BZRs in the non-infarcted cerebral cortex is associated with poor performance on the WCST in the non-disabled patients with unilateral atherosclerotic ICA or MCA disease and no cortical infarction. The BZR index of the ACA territory in the hemisphere affected by arterial disease was significantly higher in WCST-abnormal (low categories achieved for their age) patients than in WCST-normal patients, whereas the BZR index of the MCA territory was significantly different between the two groups when patients with left arterial disease were separately analysed.

This study included ICA and MCA diseases, which may have different impacts on CBF in the ACA territory. Twelve of the 17 studied patients with MCA disease had occlusion of the MCA, in which blood flowing through the ACA is redistributed via the leptomeningeal vessels to compensate for the reduced flow in the MCA, resulting in reduced flow in the ACA territory.[30] Therefore, reduced flow can cause selective neuronal damage in the ACA territory as well as the MCA territory in both patients with MCA disease and ICA disease. The difference in the BZR index between the hemisphere affected by arterial disease and the non-affected hemisphere was found in the ACA and MCA territories only, indicating that the change in the territory of BZRs was primarily in the area of the affected artery (ICA/MCA). Furthermore, a decrease in the BZRs was associated with an increase in the OEF in the ACA and MCA territories of the affected hemisphere, suggesting that selective neuronal damage may be related to haemodynamic compromise because of ICA or MCA disease.[7] Thus, the major finding of the present study was that this decrease in the BZRs in the ACA and MCA territories due to ICA or MCA disease was associated with poor performance of the WCST.

The association of cortical neuronal damage in the ACA territory with poor performance on the WCST is consistent with the results of previous studies.[31–36] The WCST assesses the executive functions, which are primarily mediated through the frontal lobes, although an extensive neural network influences the performance on the WCST.[31–33] Among patients with single focal lesions, patients with lesions in the superior medial frontal lobe, which is included in the ACA territory, were reported to have the most severely impaired WCST measures.[34] Functional neuroimaging studies have suggested the role of the medial frontal cortex in performance monitoring, leading to performance adjustments in subsequent trials.[35] Among the medial frontal regions, the anterior cingulate cortex may be engaged in the implementation of attentional control that is involved in the WCST.[33] Lesions in the anterior cingulate sulcus cortex in non-human primates impair active reference to recent choice outcomes during rule-based decision-making,[36] which may be the primary reason for the increase in both perseverative and non-perseverative errors in the patients studied.

The association of cortical neuronal damage in the MCA territory with poor performance on the WCST was apparent in patients with left arterial disease. The WCST method used in this study needs verbal mediation during the performance. Therefore, the language-related left hemisphere may contribute more than the right hemisphere to the performance of the WCST used here. Among subregions in the MCA territory, the mid-dorsolateral prefrontal region (the rostral part of the middle frontal gyrus) may be engaged in monitoring the information in the working memory, which is essential for performance on the WCST.[36] Cognitive set shifting is mediated by this region, and a lesion in this region may lead to an increase in perseverative errors.[31–33]

Table 6 Characteristics of symptomatic and asymptomatic patients

	Symptomatic		Asymptomatic	
	Abnormal	Normal	Abnormal	Normal
No of patients	23	13	16	8
Benzodiazepine receptor index (%)				
MCA territory (affected)	41.1±36.4	25.9±32.8	23.4±19.1	23.4±22.6
ACA territory	21.2±19.0*	11.0±16.8	16.0±13.4	9.4±11.3
Cerebral blood flow (ml/100 g/min)				
MCA territory	40.3±7.0	44.1±9.3	45.8±7.8	46.7±8.3
ACA territory	43.2±8.1	46.0±10.1	48.1±8.5	49.4±11.4
Cerebral metabolic rate of oxygen (ml/100 g/min)				
MCA territory	3.61±0.38	3.61±0.34	3.55±0.49	4.10±0.55
ACA territory	3.74±0.40	3.60±0.36	3.63±0.41	4.29±0.72
Age (years)	65±9	62±11	66±6	60±8
Sex, male/female (n)	14/9	10/3	14/2	7/1
Arterial disease, internal carotid artery/ MCA (n)	15/8	8/5	13/3	7/1
Side of disease, left/right (n)	11/12	6/7	8/8	3/5
Subcortical infarction (n)	23	12	8	2
Size (mm^2)	197±225	182±204	52±90	76±211
Frontal infarction (n)	16	10	5	0
Wisconsin Card Sorting Test parameters (n)				
Categories achieved	1.7±0.9*	4.3±1.1	0.8±0.9*	4.7±1.0
Total errors	28.2±8.6*	16.4±4.5	29.2±5.1*	15.1±3.7
Perseverative errors (Nelson)	12.4±9.9*	3.6±3.4	9.2±4.2*	3.3±2.3

Values are mean±SD.
*$p<0.05$, Mann–Whitney U test.
Abnormal, low categories achieved on the Wisconsin Card Sorting Test; ACA, anterior cerebral artery; MCA, middle cerebral artery.

Although the value of the BZR index in the MCA territory was higher than that in the ACA territory, in the patient sample studied, the association of the BZR index in the MCA territory with poor performance on the WCST was worse than that in the ACA territory. This may be partly because the regional distribution of ischaemic damage in the MCA territory in patients with atherosclerotic ICA or MCA disease may be rather variable.

The BZR index, CBF and $CMRO_2$ reflect different aspects of ischaemic changes in the brain in the case of patients with atherosclerotic ICA or MCA disease. A decrease in the CBF may be caused not only by a decrease in perfusion pressure but also secondarily by a decrease in the $CMRO_2$.[37] A decrease in $CMRO_2$ is one result of ischaemic damage to the tissue and can also be caused by a decrease in neural input from distant regions with fibre connections.[38] Patients with subcortical infarction may experience a decrease in cortical $CMRO_2$ through deafferentation of thalamocortical projections that is not accompanied by a decrease in cortical BZRs.[7] As shown in the case of crossed cerebellar diaschisis, a simple decrease in neural input may not necessarily be accompanied by apparent neurological deficits in the target region. In patients with chronic atherosclerotic ICA or MCA disease and subcortical infarcts, the BZR index may most accurately indicate the degree of ischaemic damage in the target regions among the three variables. This may explain why the BZR index correlated best with poor performance on the WCST in the present study, although the normalisation to the mean cerebellar value and the calculation of pixel-by-pixel Z scores for the BZR index might have contributed to the good correlation.

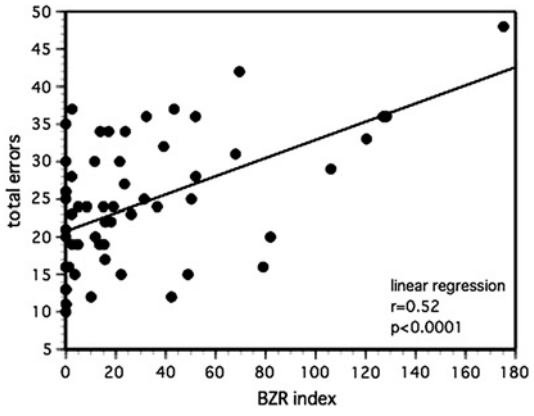

Figure 2 Scatter diagram plotting the benzodiazepine receptor (BZR) index for the anterior cingulate gyrus for the hemisphere with arterial disease against the total number of errors on the Wisconsin Card Sorting Test.

Patients with atherosclerotic ICA or MCA disease and ipsilateral TIA or stroke can have lasting cognitive impairment, despite the recovery of focal neurological deficits.[39] One of the causes of this cognitive impairment may be severe, chronic haemodynamic impairment,[39 40] which may increase the risk of selective neuronal damage.[7] In some patients with chronic and potentially reversible global ischaemia and mild neuronal damage, vascular reconstruction surgery may improve perfusion and cognitive impairment.[40 41] However, if extensive neuronal damage occurs, and low perfusion is accompanied by reduced metabolism, any therapeutic effects of improved perfusion are mitigated.[40] In patients with stroke with haemodynamic compromise, intensive treatment for improvement of perfusion and protection of the neurons should be administered in the initial stages of infarction to minimise the occurrence of selective neuronal damage and cognitive impairment, although a causal relationship between selective neuronal damage and cognitive impairment should be confirmed during follow-up studies.[14]

This study has some methodological limitations. We could not perform a correction for partial volume effects, because MRI was

studied only for clinical purposes. To minimise the effect of cortical atrophy, we used the surface projection technique in 3D-SSP, which is considered to be the best method in this case. Although we created FMZ-BP parametric maps, the normalisation to the mean cerebellar value was needed for the subsequent analysis, because interindividual variations of absolute whole-brain values for FMZ BP were large. To quantitatively assess the degree of FMZ BP reduction in each patient, we calculated the BZR index. Although we extracted pixels with Z scores >2 as abnormal pixels, the issue of multiple comparisons was not taken into account, and the new measurement of the BZR index may be biased. In previous studies, the BZR index was shown to be stable in the sequential evaluations of normal subjects and to be associated with haemodynamic compromise in patients with ICA or MCA disease.[7] In the present study, we showed that the BZR index was correlated with clinical variables. Although we consider the BZR index a useful quantitative measure of decreases in BZR, further studies should be done to validate it.

In conclusion, in atherosclerotic ICA or MCA disease, a decrease in BZRs in the non-infarcted cerebral cortex affected by arterial disease is associated with poor performance on the WCST. Selective neuronal damage may contribute to the occurrence of cognitive impairment in patients with atherosclerotic ICA or MCA disease.

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