Cerebral Oxygen Metabolism and Neuronal Integrity in Patients With Impaired Vasoreactivity Attributable to Occlusive Carotid Artery Disease

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**Background and Purpose**—It is still unclear that impaired cerebrovascular reactivity (CVR) to acetazolamide is comparable to elevated oxygen extraction fraction (OEF) on positron emission tomography (PET) in patients with occlusive carotid diseases. Therefore, in this study, the authors aimed to clarify whether OEF is elevated in all patients with reduced cerebral blood flow (CBF) and CVR (type 3) on single photon emission computed tomography (SPECT), and, if not, to specify the underlying pathophysiology of type 3 but normal OEF.

**Methods**—This study included 46 patients who had decreased CBF and CVR on N-isopropyl-p-123I-iodoamphetamine SPECT in the ipsilateral middle cerebral artery area attributable to occlusive carotid diseases. Hemodynamic and metabolism parameters were determined in all patients by 15O-gas PET, and neuronal integrity was evaluated in 19 patients using 11C-flumazenil (FMZ) PET.

**Results**—OEF was significantly elevated in 20 (43.5%) of 46 type 3 patients. Another 26 type 3 patients had normal OEF. Regression analysis showed that OEF significantly correlated with cerebral metabolic rate for oxygen and 11C-FMZ binding potential but not with other parameters. Subcortical infarction had no significant effect on OEF values.

**Conclusions**—The results strongly suggest that type 3 patients with reduced CBF and CVR may be divided into 2 pathophysiologically different subgroups: misery perfusion attributable to hemodynamic compromise and matched hypometabolism attributable to incomplete infarction. Type 3 but normal OEF may represent a transition stage from misery perfusion to matched hypometabolism. *(Stroke. 2006;37:393-398.)*

**Key Words:** acetazolamide ■ cerebral ischemia ■ flumazenil ■ metabolism ■ oxygen

There is increasing evidence that hemodynamically compromised patients with internal carotid artery (ICA) occlusion are at higher risk for subsequent ischemic stroke. Over these 20 years, an elevated oxygen extraction fraction (OEF) determined by positron emission tomography (PET) has been believed to represent critical reduction of cerebral perfusion pressure, named as “misery perfusion” or “stage II.”1,2 Recent statistical analyses have proven that an elevated OEF can be an independent risk factor for subsequent ischemic stroke in patients with occlusive carotid artery disease.3–5

Alternatively, cerebrovascular reactivity (CVR) to CO2 or acetazolamide has also been used to assess cerebral perfusion reserve in patients with occlusive carotid diseases because single photon emission computed tomography (SPECT) or cold xenon computed tomography (CT) is more widely available and can be done at lower costs than PET. Recent studies have proven that quantitative measurements of cerebral blood flow (CBF) and CVR can also be a predictor for subsequent ischemic stroke in patients with ICA or middle cerebral artery (MCA) occlusion. Thus, Kuroda et al (2001) reported that relative risk conferred by reduced CBF and CVR (type 3) was 8.0 (95% CI, 1.9 to 34.4) for ipsilateral stroke.6 Subsequently, Ogasawara et al also reported similar results.7 Based on these observations, SPECT has been expected to identify misery perfusion or stage II more easily than PET if CVR is comparable to OEF.8

However, it is still controversial whether impaired CVR is directly linked to OEF elevation in patients with occlusive carotid artery diseases or not. Thus, previous studies have reported a significant correlation between OEF and CVR to acetazolamide or CO2.9–14 However, the number of patients included in these studies was not so large, and their hemodynamic and metabolic parameters varied widely among the subjects. On the other hand, recent study has shown that ≈40% of patients with reduced CVR have normal OEF when both parameters are evaluated in each patient.15 The issue is...
quite important because there may be a significant difference in sensitivity for detecting the patients at higher risk for subsequent stroke between CVR and OEF.

On the other hand, $^{11}$C-flumazenil (FMZ) PET has been accepted as a noninvasive, variable tool to investigate neuronal integrity because FMZ is a specific ligand to the central type of benzodiazepine receptors that are exclusively localized in the neurons. Recent studies have shown that $^{11}$C-FMZ PET can detect ischemia-induced selective neuronal necrosis that is not visible on either CT or MRI.6,16,17

Therefore, in this study, the authors aimed to clarify whether OEF is elevated in all patients who are diagnosed as type 3 on SPECT, and, if not, to specify the underlying pathophysiology of normal OEF in spite of type 3. For this purpose, the authors measured the parameters for oxygen metabolism and for neuronal integrity in type 3 patients with occlusive, using $^{15}$O-gas and $^{11}$C-FMZ PET, respectively.

Subjects and Methods

Patients

The present study included a total of 46 patients who were admitted to our hospital between January 1999 and December 2004. All of them met the following criteria: (1) severe stenosis (>90%) or occlusion of the ipsilateral ICA or MCA; (2) no or, if any, small infarction on MRI; and (3) reduced CBF and CVR to acetazolamide in the ipsilateral MCA territory on $^{[123]}$I-iodoamphetamine (123I-IMP) SPECT (see below). There were 36 men and 10 women with a mean age of 68.2 years (range 48 to 79 years). Their clinical symptoms included transient ischemic attack or amaurosis fugax in 18 patients and minor completed stroke (Rankin score 1 or 2) in 25. The other 3 patients were asymptomatic. Digital subtraction angiography showed ICA occlusion in 27 patients, ICA severe stenosis in 8, MCA occlusion in 5, and MCA severe stenosis in 6. All studies were performed 24 to 7 weeks after the last ischemic episode because the studies in an earlier period might affect the correct interpretation of the data.18

SPECT Measurements

All patients were scanned with a triple-head γ camera (GCA-9300/DI; Toshiba) to determine CBF and CVR to acetazolamide, as described previously.16 Briefly, quantitative blood flow was determined by using the $^{123}$I-IMP injection and single-scan autoradiographic technique. CBF was quantitatively measured before and 15 minutes after intravenous injection of 10 mg/kg acetazolamide on the separate days with an interval of 2 to 3 days. To evaluate cerebral hemodynamics, 10-mm diameter circular regions of interest (ROIs) were symmetrically placed in the ipsilateral and contralateral MCA territories. As described previously,16,19 CVR to acetazolamide was quantitatively calculated as: CVR (%) = 100 × (CBFint - CBFrest)/CBFint, where CBFint and CBFrest represent CBF before and after intravenous injection of acetazolamide, respectively. Normal control values of CBF (mean ± SD = 38.1 ± 5.4 mL/min per 100 g) and CVR (30.0 ± 8.0%) in the MCA territory were obtained from 10 normal volunteers free of cerebrovascular disease. The values were rated as reduced when any of them were less than mean – 2 SD. Thus, in the current study, patients were judged as type 3 when CBF was <27 mL/min per 100 g and CVR was <14%.16

PET Measurements

All patients were scanned with ECAT EXACT HR+ (Siemens) as described previously.16 The intervals between SPECT and PET measurements were within 2 weeks. One-minute inhalation of $^{15}$O-02 (2 GBq/min) followed by 3-minute static scanning and 3-time arterial blood sampling were performed to measure cerebral blood volume (CBV). After 15-minute inhalation of $^{15}$O-O2 (0.5 GBq/min), a steady-state O2 image was scanned and 3-time arterial blood sampling was performed for 5 minutes to measure OEF and cerebral metabolic rate for oxygen (CMRO2). Finally, to determine CBF, steady-state CO image was scanned and 3-time arterial blood sampling was performed for 5 minutes after 15-minute inhalation of $^{15}$O-CO2 (0.5 GBq/min). Normal PET values were obtained from 10 volunteers: CBF, 44 ± 4 mL/min per 100 g; CMRO2, 3.3 ± 0.6 mL/min per 100 g; CBV, 3.7 ± 0.7 mL/min, and OEF, 0.43 ± 0.05 (mean ± SD). Each PET parameter was obtained using 10-mm diameter circular ROIs. The values were rated as decreased when any of them were less than mean – 2 SD and rated as increased when any of them were more than mean + 2 SD.

The dynamic FMZ PET was studied in 19 of 46 patients at the same time that $^{15}$O-gas PET was performed, as reported previously.16 Briefly, the injected dose of $^{11}$C-FMZ was 370 MBq for each patient. The binding potential (BP) images were calculated pixel by pixel using the reference tissue model.20

Data Analysis

To evaluate various parameters obtained from $^{123}$I-IMP SPECT, $^{15}$O-gas PET, and $^{11}$C-FMZ PET, the SPECT and PET images were automatically coregistered to axial T1-weighted MRI images. The SPECT, PET, and MRI images were registered using fully automatic multimodality image registration algorithm on Unix-based workstation (Indigo 2; SGI Inc.).21

All data were expressed as mean ± SD. The data between 2 groups were compared by use of χ² test or paired t test as appropriate. Differences with a P value of <0.05 were considered statistically significant.

Results

$^{15}$O PET Parameters

CBF, CBV, CMRO2, and OEF in type 3 patients are shown in the Table. There were significant differences in CBF, CMRO2, and OEF between the ipsilateral and contralateral MCA areas. However, there was no significant difference in CBV between them.

Relationships between OEF and other PET parameters were analyzed in the ipsilateral hemispheres (Figure 1). There was no significant correlation between OEF and CBF ($R^2=0.001$;
P = 0.841), between OEF and CBV ($R^2 = 0.041; P = 0.1794$), or between OEF and mean transit time (MTT; $R^2 = 0.023; P = 0.3169$). On the other hand, there was significant, positive correlation between OEF and CMRO$_2$ ($R^2 = 0.081; P = 0.006$).

Then the values of OEF, CMRO$_2$, and CBV were evaluated in each patient. Although OEF was significantly higher in the ipsilateral MCA area than in the contralateral side, OEF was significantly elevated in only 20 (43.5%) of 46 patients. OEF was kept within normal limits in the other 26 patients (Figure 2).

CMRO$_2$ was significantly higher in patients with elevated OEF than in those with normal OEF: 2.26 ± 0.41 and 1.78 ± 0.42 mL/100 g per minute, respectively ($P = 0.0002$; Figure 3). Of 20 patients with elevated OEF, 14 (70%) had normal CMRO$_2$ and the other 6 (30%) had decreased CMRO$_2$ (2.1 mL/100 g per minute). On the other hand, of 26 patients with normal OEF, 7 (26.9%) had normal CMRO$_2$ and the other 19 (73.1%) had decreased CMRO$_2$. Thus, normal CMRO$_2$ was more frequently observed in patients with elevated OEF than in those with normal OEF ($P = 0.0032$; Figure 3).

There was no significant difference in CBV between patients with elevated OEF and with normal OEF: 4.4 ± 1.1 and 4.0 ± 1.1 mL/100 g, respectively ($P = 0.2357$; Figure 3). However, of 20 patients with elevated OEF, 9 (45%) had increased CBV. Of 26 patients with normal OEF, only 4 (15.4%) had increased CBV. As the result, increased CBV was more frequently denoted in patients with elevated OEF than in those with normal OEF ($P = 0.0264$; Figure 3).

**11C-FMZ Binding Potential**

To evaluate the neuronal integrity in patients with type 3 ischemia, $^{11}$C-FMZ PET was performed in 19 (41.3%) of 46 patients. The relationships between the ratio of the ipsilateral to contralateral $^{11}$C-FMZ BP and metabolic parameters were analyzed. There was a significant, positive correlation between the ratio and OEF ($R^2 = 0.507; P = 0.0006$; Figure 4). The ratio also significantly correlated with CMRO$_2$ ($R^2 = 0.324; P = 0.011$).

**MRI**

Using T2-weighted MRI, the localization of cerebral infarction was evaluated to clarify its effects on cerebral oxygen metabolism and neuronal integrity. Subcortical infarction in the ipsilateral hemisphere was found in 7 of 20 patients with elevated OEF and in 16 of 26 patients with normal OEF. There was no significant effect of subcortical infarction on OEF value in type 3 patients ($\chi^2$ test $P = 0.0743$).

**Discussion**

The present results revealed that hemodynamic and metabolic parameters in type 3 patients are not uniform, and that they
can be largely classified into 2 subgroups according to OEF value. OEF was significantly elevated in ≈40% of type 3 patients and was within normal limits in the others, indicating that type 3 is not always identical to misery perfusion or stage II. CMRO₂ was significantly higher in patients with elevated OEF than in those with normal OEF (Figure 3A) and significantly correlated with OEF (Figure 1D). Therefore, OEF may depend on the metabolic demand in the ischemic tissue.

As the next step, ¹¹C-FMZ BP and the localization of cerebral infarction were evaluated to specify the underlying pathophysiology of CMRO₂ reduction in the area with type 3 but normal OEF. Subcortical infarction in the ipsilateral hemisphere was not directly related to type 3 but normal OEF, although previous reports suggested its involvement. However, there was a significant correlation between OEF and the ¹¹C-FMZ BP in type 3 patients. Because γ-amino-butyric acid receptors are abundant in the cortex and sensitive to ischemic damage, the specific ligand to their subunits, the central type of benzodiazepine receptors, has been used as a marker of preserved morphological integrity. Garcia et al emphasized the importance of selective neuronal necrosis (incomplete infarction) in human stroke as a pathologic entity. Recently, the authors demonstrated that CMRO₂ and ¹¹C-FMZ BP were reduced to ≈80% of the contralateral side, but there was no significant side-to-side difference in CBV and OEF in patients with reduced CBF and normal CVR (type 4) and concluded that type 4 represents oxygen hypometabolism attributable to ischemia-related selective neuronal damage. Previous studies have shown that the patients with type 4 may not be at high risk for subsequent stroke when medically treated. The PET parameters in patients with type 3 ischemia but normal OEF are quite similar to those in the patients with type 4.

Based on these observations, type 3 may include 2 pathophysiologically different conditions: misery perfusion (or stage II ischemia) attributable to hemodynamic compromise, and matched hypometabolism attributable to incomplete infarction. Although the authors have simply graded cerebral hemodynamics of the patients with occlusive carotid artery diseases into 4 types, type 3 should be subdivided into “true type 3,” with elevated OEF, and “type 3.5,” with normal OEF, in discussing their pathophysiology and long-term prognosis. It is obscure why CVR is impaired in patients with type 3 but normal OEF. As Yamauchi et al pointed out, such

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**Figure 2.** Plot of OEF values in the ipsilateral and contralateral hemisphere of type 3 patients. Dotted line indicates the upper limit of normal OEF value.

**Figure 3.** Plots of ipsilateral values of CMRO₂ (A) and CBV (B) in patients with elevated and normal OEF. Dotted lines indicate the lower limit of normal CMRO₂ value (A) and the upper limit of normal CBV value (B).

**Figure 4.** Regression analysis of the relationship between OEF and the BP for ¹¹C-FMZ in 19 type 3 patients.
patients may have complex hemodynamic and metabolic changes in response to both reduced perfusion pressure and ischemic tissue damage.\textsuperscript{14} Present results mirror previous descriptions, that is, using \textsuperscript{133}Xe inhalation method and SPECT, the authors divided 32 patients with ICA occlusion into 4 types and serially measured CBF and CVR after superficial temporal artery to MCA anastomosis. Seven patients were diagnosed as having type 3 before surgery. The CVR normalized in all type 3 patients, suggesting postoperative improvement of cerebral perfusion reserve. But CBF returned to normal range in 3 (42.8\%) of 7 type 3 patients. As the result, SPECT parameters altered from type 3 to type 4 in other 4 patients.\textsuperscript{18} Furthermore, they recently assessed long-term prognosis of 77 patients who were medically treated because of ICA or MCA occlusion. Of 11 type 3 patients, 4 (36.4\%) developed ipsilateral ischemic stroke during follow-up periods.\textsuperscript{5} The present results may explain these variations in type 3 patients.

However, as recent studies have clarified, hemodynamic and metabolic responses to reduced perfusion pressure are not so simple. Patients with “classic” misery perfusion (elevated OEF and CBV) are at highest risk for subsequent stroke.\textsuperscript{22} However, CBV changes widely vary in patients with occlusive carotid artery disease. This study also showed that CBV widely varied in spite of OEF values. Further studies would clarify the CBV responses to chronic cerebral ischemia more precisely.

This study showed that type 3 is not equal to misery perfusion. However, SPECT and acetazolamide test are still useful modalities because they can simply select the patients at higher risk for subsequent ischemic stroke at lower costs than PET, as described previously.\textsuperscript{6,7} Thus, it is very valuable to establish the methodology to detect misery perfusion more efficiently with the use of SPECT because PET is not widely available. Based on a significant linear correlation between OEF and \textsuperscript{11}C-FMZ BP in this study, the authors propose to evaluate whether \textsuperscript{123}I-iodomazenil (IMZ) SPECT can detect misery perfusion or stage II ischemia in type 3 patients more efficiently.\textsuperscript{12} \textsuperscript{123}I-IMZ is an alternative benzodiazepine receptor ligand for SPECT and has been reported that a reduction of its binding reflects oxidative hypometabolism caused by neuronal damage in hemodynamically impaired areas in patients with cerebral vascular disease.\textsuperscript{23–25} Therefore, SPECT may be able to identify the patients with misery perfusion by measuring CVR and \textsuperscript{123}I-IMZ binding, if the results on \textsuperscript{123}I-IMZ SPECT are comparable to those on \textsuperscript{11}C-FMZ PET in patients with occlusive carotid artery diseases.

**Conclusion**

Previous studies have shown that type 3 (reduced CBF and CVR) as well as elevated OEF is statistically independent predictors for subsequent stroke in patients with occlusive carotid artery diseases.\textsuperscript{3,4,6,26} However, this study clearly showed that OEF was elevated in \textasciitilde 40\% of patients with reduced CBF and CVR (type 3). Significant, positive linear relationships were observed between OEF and CMRO\textsubscript{2} and between OEF and \textsuperscript{11}C-FMZ BP. Type 3 may include 2 pathophysiologically different conditions: misery perfusion (or stage II) attributable to hemodynamic compromise and matched hypometabolism attributable to incomplete infarction. Further studies would be necessary to define the SPECT parameter to select the patients at higher risk for subsequent stroke more specifically.

**References**


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Stroke. 2006;37:393-398; originally published online December 29, 2005; doi: 10.1161/01.STR.0000198878.66000.4e

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