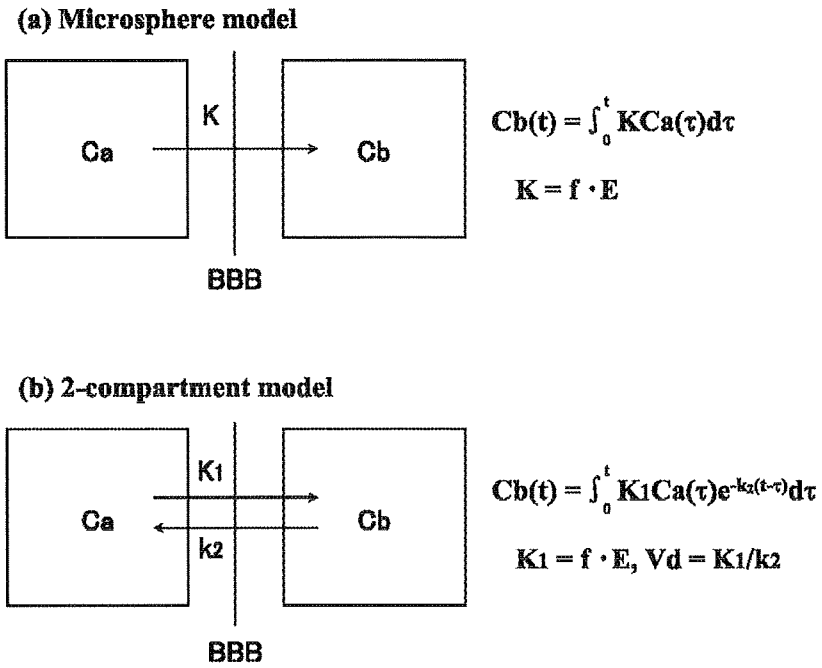

図2．蓄積型脳血流トレーサーを用いたCBFの定量法（コンパートメント解析）
(a) マイクロスフェアーモデル
(b) 2-コンパートメントモデル
BBB：血液脳関門
K（K1）：トレーサーの血液から脳への移行速度定数
k2：トレーサーの脳から血液への移行速度定数
Vd = K1/k2：分布容積
f：局所脳血流量
Ca（t）：t時間後の血中放射能（入力関数）
Cb（t）：t時間後の脳局所放射能
E：初回循環摂取率（E = 1 − ePS/f）（PS：permeability-surface area product）

脳循環予備能が算出される．次いでピクセル毎に安静時脳血流量と算出された脳循環予備能から血行力学的脳虚血の重症度が決定される．いずれの指標も8方向からの3次元脳表画像として定位的に画像化される（図4）．本法では，脳表における血行力学的脳虚血の各重症度の分布を視覚的に判定できるとともに，予め設定された血管支配領域内のピクセル数に対して各重症度のピクセル数の割合を算出し，数量的に判定をすることも出来る．本解析法により，脳血流SPECTの定位定量的解析が可能となり，血行力学的脳虚血の重症度評価の判定精度の改善とともに画像診断の標準化が進むものと考えられる．

Ⅲ　Bz受容体SPECT統計画像解析

^{123}I-Iomazenil（IMZ）を用いた中枢性Bz受容体のSPECTによる分布画像は，半定量的な皮質神経細胞の分布を表す．中枢性Bz受容体は，大脳皮質に広範に存在するGABA系抑制シナプスの一部をなし，その分布画像は，皮質神経細胞の脱落の程度を表すマーカーとして臨床的意義を有する[7–9]．IMZ-SPECTの解析法としては，当初患側ROI係数値の対健側比などが用いられたが，最近では，診断精度の向上のために3D-SSPなどの統計画像解析が導入されている．IMZ-SPECTの診断適応は，現在のところ外科的切除が適応となるてんかん焦点を同定する画像診断法に限られているが，今後は以下のような臨床応用が想定される．第一に血栓溶解療法などの脳虚血後再灌流に伴う不完全脳梗塞[10]の診断，第二にmisery perfusionや血行力学的脳虚血の判定精度の改善，第三にもやもや病における慢性脳虚血と高次脳機能障害の診断[11]，第四にMRIなどで確認できない脳外傷と高次脳機能障害（前頭葉内側障害）の評価，などへの応用である．

不完全脳梗塞の診断

虚血性ペナンブラ領域の血行再開に関するIMZ-SPECTを用いた臨床研究により，血行再開後のCT/

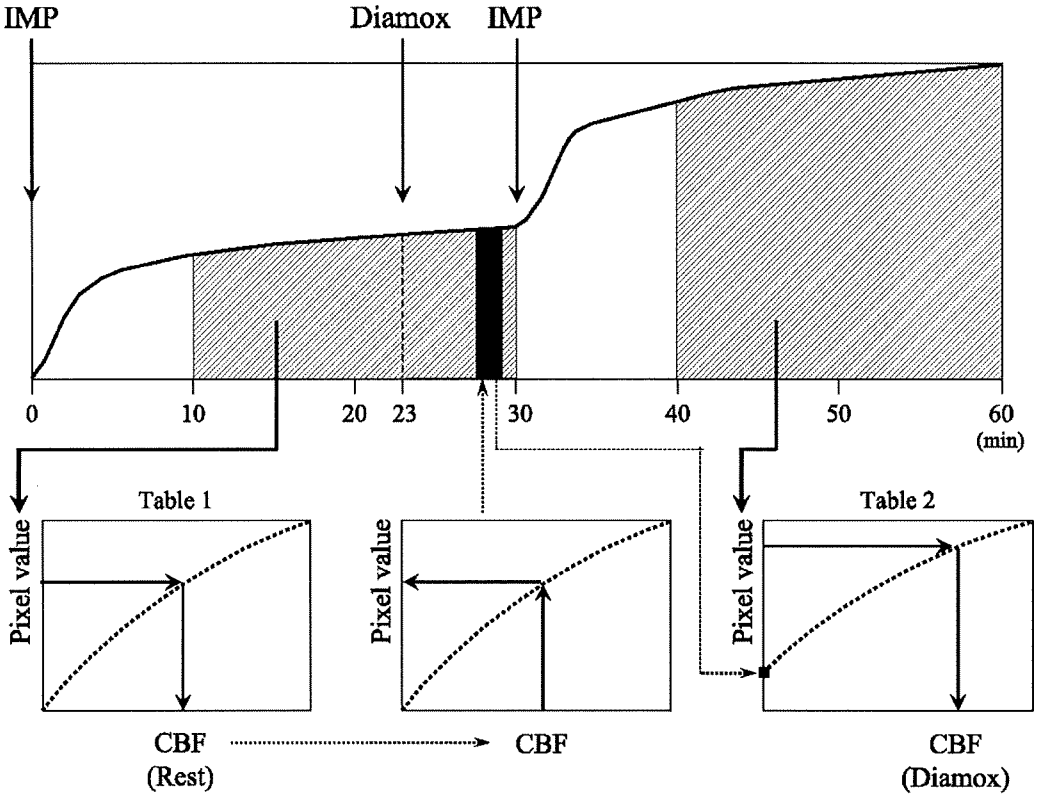

図3. Dual table ARG 法の理論

等量のトレーサーを用いて安静時と acetazolamide 負荷時の SPECT 計数値を連続的に求め，それぞれに対して SPECT 計数値と脳血流量の関係を表す table を作成することにより，安静時と acetazolamide 負荷時脳血流量の定量画像を得る方法である．安静時脳血流量の画像化では，IMP-ARG 法に準じて決定された入力関数を用いて SPECT 計数値（左の斜線部分から得られる pixel value）と安静時脳血流量との関係が table 化され（左下の Table 1），この Table を参照して各 pixel の計数値が安静時脳血流量に変換される．Acetazolamide 負荷時血流量の画像化では，安静時と同一の入力関数を用いるが，各 pixel での安静時終了時の SPECT 計数値を算定した上で SPECT 計数値（右の斜線部分から得られる pixel value）と acetazolamide 負荷時脳血流量との関係が table 化され（右下の Table 2），この Table を参照して各 pixel の計数値が acetazolamide 負荷時脳血流量に変換される．

MRI において変化がない領域で慢性期に中枢性 Bz 受容体の減少が認められ，脳虚血後の皮質神経細胞の選択的脱落（不完全脳梗塞）の発生が画像化されている[10]．不完全脳梗塞の発生については，すでに実験的な急性脳虚血—血流再開モデルにおいて明らかにされている[12]．図5に急性脳虚血発作後に不完全脳梗塞と診断された症例の MRI と IMZ-SPECT，図6にその 3D-SSP 解析の結果を呈示する．急性期に脳虚血に陥った領域のうち MRI で脳梗塞巣が認められない領域でも，慢性期には脳血流とともに中枢性 BZR の減少が認められ，同領域の脳虚血によって皮質神経細胞が選択的に脱落し，不完全脳梗塞が生じたと考えられる．臨床病態診断としての不完全脳梗塞の概念は，血栓溶解療法の効果判定のみならず，今後，血栓溶解療法後の高次脳機能障害などを判定する上でもきわめて重要と考えられる．

Misery perfusion の診断

血行力学的脳虚血 Stage II と評価された領域に関する IMZ-SPECT を用いた臨床検討では，中枢性 BZR が低下している場合と保持されている場合があることが判明している．これらは脳血流 SPECT の SEE 解析により定位的に捉えられる血行力学的脳虚血 Stage II の領域と IMZ-SPECT の 3D-SSP の Z-score 解析画像とを比較することにより明らかとなる．図7に 72 歳男性，一過性脳虚血発作で発症した右内頸動脈狭窄症例（アテローム血栓性）の脳血流 SPECT 定量測定の SEE 解析と IMZ-SPECT の 3D-SSP（Z-score）解析の結果を示す．前者では右前大脳動脈・中大脳動脈

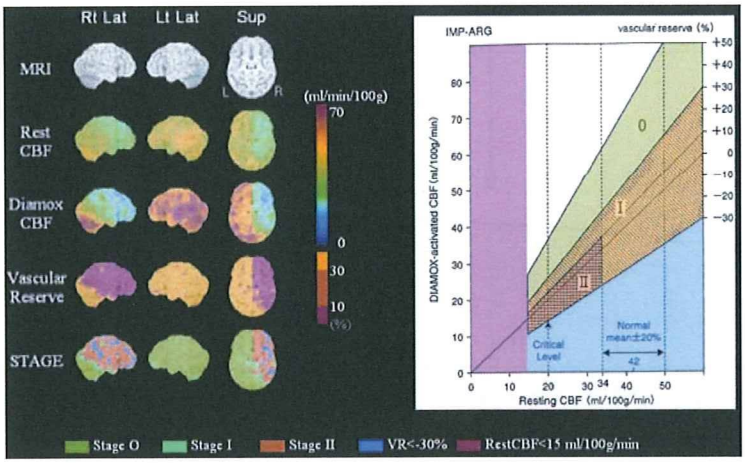

図4. Stereotactic extraction estimation（SEE）解析による血行力学的脳虚血の定位的重症度評価と血行力学的脳虚血の評価基準

(右)SEE解析では，被検者の安静時およびacetazolamide負荷時の脳血流SPECT定量画像データから脳表血流量を抽出し，これを定位脳座標系（Talairachの標準脳）に変換することにより各ピクセルにおける脳循環予備能が算出される．次いで，ピクセル毎に安静時脳血流量と算出された脳循環予備能から血行力学的脳虚血の重症度が決定される．上段から，標準脳のMRI，安静時脳血流量，acetazolamide負荷時脳血流量，脳循環予備能，血行力学的脳虚血のStageが8方向からの3次元脳表画像として定位的に画像化される．

(左) 安静時およびacetazolamide負荷時脳血流定量測定による血行力学的脳虚血の定量的重症度評価（斜線の傾きが脳循環予備能の程度を示す）

Stage 0：脳循環予備能：正常（30% <）

Stage I：脳循環予備能：低下（10% <，≦ 30%），あるいは
安静時脳血流量：正常範囲（正常平均値の80% ≦）

Stage II：脳循環予備能：喪失（≦ 10%），かつ
安静時脳血流量：低下（<正常平均値の80%）

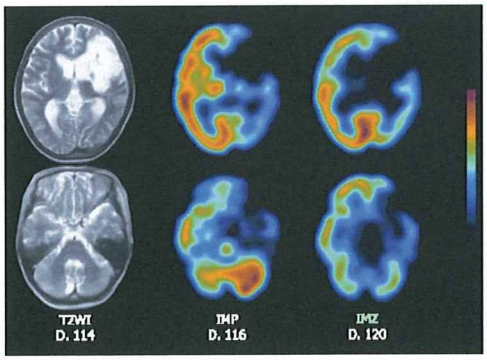

図5. 急性脳虚血発作後の不完全脳梗塞の画像診断（文献10より引用）

61歳女性．突然の右片麻痺，失語症にて発症した左頭蓋内内頸動脈の閉塞後再開通例(脳塞栓症)．慢性期のMRIでは，左前頭葉および基底核部に脳梗塞所見が認められ(左列)，神経学的には全失語であった．IMP SPECTでは，MRIで脳梗塞巣が認められない左側頭葉および対側小脳の血流が低下していた（中列）．しかし，IMZ SPECTでは左側頭葉の集積は低下していたが，小脳の集積には左右差がみられなかった（右列）．中枢性BZRの減少を伴う左側頭葉の血流低下は，対側小脳の血流低下とは異なり，脳梗塞巣からの遠隔効果（diaschisis）によるものではなく，脳虚血によって皮質神経細胞が選択的に脱落し，不完全脳梗塞が生じた結果と考えられた．

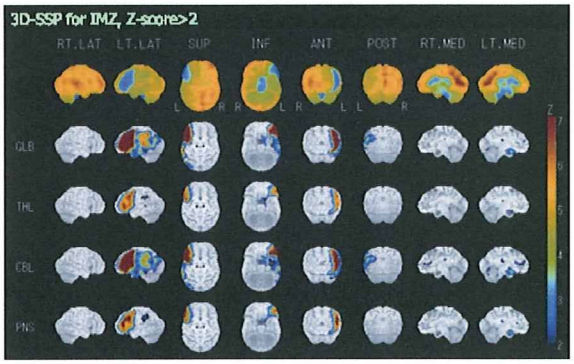

図6. 図5の症例のIMZ-SPECT 3D-SSP（Z-score）解析（上段より，IMZの分布，全脳・視床・小脳・橋で正規化したZ-score画像）

急性期に脳虚血に陥った左内頸動脈領域のうちMRIで脳梗塞巣が認められない頭頂・側頭領域でも，慢性期には脳血流とともに中枢性BZRの減少が認められ，同領域の脳虚血によって皮質神経細胞が選択的に脱落し，不完全脳梗塞が生じたと考えられた．

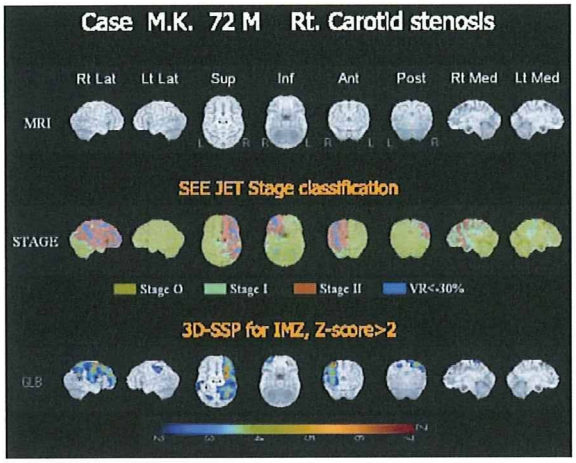

図7. 血行力学的脳虚血に伴う不完全脳梗塞のSPECT画像解析
一過性脳虚血発作（左片麻痺）にて発症した72歳男性．右頸部内頸動脈高度狭窄症（アテローム血栓性）と診断された．脳血流（IMP）-SPECT SEE解析では，右前大脳動脈・中大脳動脈領域に広範な血行力学的脳虚血 Stage IIが認められたが，IMZ-SPECT 3D-SSP（Z-score）解析では主として前大脳動脈と中大脳動脈の境界領域に，皮質神経細胞の脱落領域（不完全脳梗塞）が認められた．

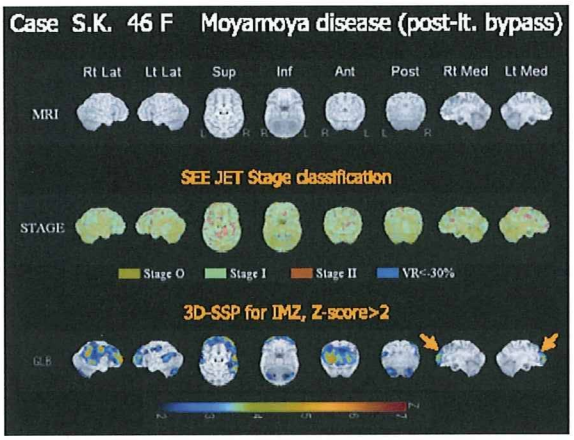

図8. もやもや病の高次脳機能障害例のSPECT画像解析
一過性脳虚血発作と頭痛にて発症した46歳女性，もやもや病と診断され血行再建術が施行された．術後，新たな脳梗塞は認められなかったが，神経心理学検査にて記憶障害，注意障害，遂行機能障害，などが認められた．IMP-SPECT SEE解析ではほとんどの領域がStage 0～Iと評価され，Stage IIはごく一部に止まったが，IMZ-SPECT 3D-SSP（Z-score）解析では右側の中大脳動脈領域の一部に加えて，両側前頭葉の前方内外側に皮質神経細胞の脱落領域（不完全脳梗塞）が認められた．

領域に広範な血行力学的脳虚血Stage IIが認められ，後者では主として右前大脳動脈・中大脳動脈の境界領域に皮質神経細胞の脱落が認められる．

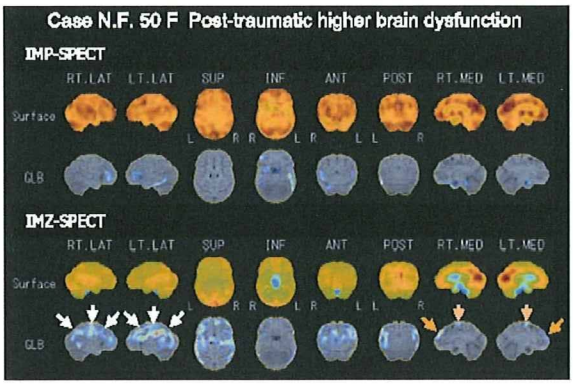

図9. 脳外傷後高次脳機能障害例のSPECT画像解析
7年前の交通事故にて高次脳機能障害と診断された50歳女性．MRIでは器質的脳損傷が明確でなかったが，神経心理学検査にて知能障害（WAIS III:境界域より下）に加えて，記憶障害，注意障害，遂行機能障害，などが認められた．IMP-SPECT 3D-SSP（Z-score）解析では，明らかな脳血流の低下領域は見られなかったが，IMZ-SPECT 3D-SSP（Z-score）解析では両側の前頭葉・頭頂葉外側に加えて，両側前頭葉の前方内側に脳皮質神経細胞損傷領域（Z-score＞2の低下を示すピクセルの集合領域）が認められた．

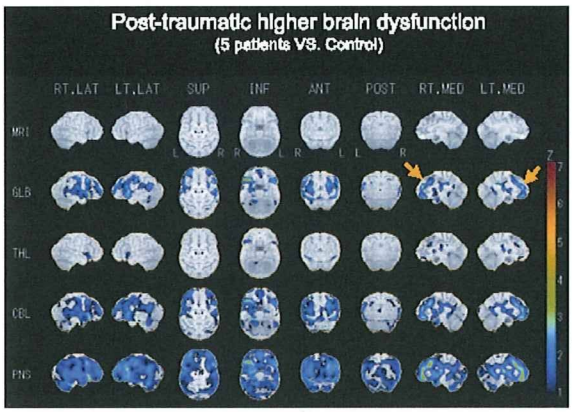

図10. 脳外傷後高次脳機能障害例のSPECT画像解析
MRIなどで器質的脳損傷が明確でなく，神経心理学的検査にて記憶障害，注意障害，遂行機能障害，社会的行動障害などの『高次脳機能障害』が確認された5症例と健常群との群間比較（3D-SSP解析）により，両側前部帯状回皮質に有意な脳皮質神経細胞損傷領域（Z-score＞1の低下を示すピクセルの集合領域）が確認された．

血行力学的脳虚血Stage IIについては，IMZ-SPECT統計画像解析により，遷延性の皮質神経細胞脱落が見られないか軽度であることを追加することにより，Misery perfusion[13]の診断精度を改善させる可能性もあり，今後の検討が必要である．

もやもや病に伴う高次脳機能障害の診断

記憶障害，注意障害，遂行機能障害，社会的行動障害などの認知障害が認められ，MRIなどで器質的病

変が確認されない成人もやもや病において，長期の血行力学的脳虚血が原因と思われる前頭葉の皮質神経細胞脱落(Z-score>2の低下を示すピクセルの集合領域)がIMZ　SPECT統計画像により確認された（図8）．もやもや病では，長期にわたる血行力学的脳虚血により皮質神経細胞脱落（不完全脳梗塞）が生じることが確認されており[11]，IMZ　SPECT統計画像解析は，もやもや病に伴う高次脳機能障害の診断に有用と考えられる．

脳外傷後高次脳機能障害の診断

神経心理学的検査にて記憶障害，注意障害，遂行機能障害，社会的行動障害などの『高次脳機能障害』が確認された5症例を対象としてIMZ　SPECTを施行し，3D-SSP統計画像解析を行ったところ，各症例の^{123}I-IMZ　SPECT統計画像ではいずれにおいても両側前頭葉内側に脳皮質神経細胞の損傷領域が確認された(図9)．また，5症例と健常群との群間比較により，両側前部帯状回皮質に有意な脳皮質神経細胞の損傷領域が確認された（図10）．一方，^{123}I-IMP SPECTに対する3D-SSP統計画像では同領域に有意な脳血流の低下域は認められなかった．したがって，^{123}I-IMZ SPECT統計画像は，MRIなどで器質的脳損傷が明確でない脳外傷後高次脳機能障害の診断に有用と考えられる．

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Abstract

Quantitative analysis of functional neuroimagings using SPECT

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In functional neuroimagings using SPECT, some recent development of analytic techniques for SPECT images have contributed to improve the accuracy of functional neuroimagings. In quantification of CBF image using 123-I-iodoamphetamine (IMP)-SPECT, introduction of dual table ARG (DTARG) could make 1-day serial measurement of both resting and acetazolamide-activated CBF images which overcomes measurement errors from different input functions associated with 2-days measurement of CBF images. Also, introduction of stereotactic extraction estimation (SEE) using standardized brain mapping for quantified CBF images could make stereotactic assessment of both severity of hemodynamic cerebral ischemia and its serial changes. These analytic techniques for CBF SPECT such as DTARG and SEE could improve the accuracy of SPECT measurement and diagnostic decision, and could promote the standardization of functional neuroimagings. On the other hands, central benzodiazepine receptor imaging using 123-I-iomazenil (IMZ) SPECT which clinically interpreted as the marker of cortical neuron damages could be applied not only to demonstrate incomplete brain infarction associated with cerebral ischemia, but also to estimate higher brain dysfunction associated with moyamoya disease (long-standing ischemia) or traumatic brain injury. Quantitative analysis of functional neuroimagings using SPECT could be applied for future clinical trial due to the progression of standardization techniques for SPECT images.

Key words: 123-I-iodoamphetamine (IMP), 123-I-iomazenil (IMZ), single photon emission computed tomography (SPECT), dual table ARG (DTARG), 3-dimensional stereotactic surface projections (3D-SSP)

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Brief Communication

Circulating CD34-positive cells have prognostic value for neurologic function in patients with past cerebral infarction

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Increasing evidence points to a role for circulating endothelial progenitors, including populations of CD34-positive (CD34$^+$) cells present in peripheral blood, in vascular homeostasis and neovascularization. In this report, circulating CD34$^+$ cells in individuals with a history of cerebral infarction were correlated with changes in neurologic function over a period of 1 year. Patients with decreased levels of CD34$^+$ cells displayed significant worsening in neurologic function, evaluated by the Barthel Index and Clinical Dementia Rating. These results support the hypothesis that levels of circulating CD34$^+$ cells have prognostic value for neural function, consistent with their potential role in maintaining cerebral circulation.
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Keywords: CD34; cerebral circulation; neurologic function

Introduction

Increasing evidence points to a role for circulating CD34-positive (CD34$^+$) cells in maintaining vascular homeostasis, both as a pool of endothelial progenitor cells (EPCs) and as a source of multiple growth/angiogenesis factors (Majka *et al*, 2001). Previously, we have shown accelerated neovascularization after administration of CD34$^+$ cells in an experimental model of stroke (Taguchi *et al*, 2004*b*), and observed a positive correlation between levels of circulating CD34$^+$ cells and neovascularization (Yoshihara *et al*, 2008) and regional blood flow (Taguchi *et al*, 2004*a*) in patients with chronic cerebral ischemia. In addition, we have delineated a contribution of circulating CD34$^+$ cells in support of neurologic function, presumably through their positive influence on the cerebral circulation in settings of ischemic stress (Taguchi *et al*, 2008). A role for circulating CD34$^+$ cells in vascular homeostasis has also been considered in other ischemic settings, such as myocardial (Okada *et al*, 2008) and peripheral vascular disease (Fadini *et al*, 2006*b*).

On the basis of these observations, we have hypothesized that circulating CD34$^+$ cells may contribute to the maintenance of neurologic function by enhancing cerebrovascular homeostasis in patients with a history of cerebral infarction. In this study, we have investigated the predictive value of the level of peripheral CD34$^+$ cells on neurologic function in patients with past cerebral infarction. Our results display a correlation between decreased levels of CD34$^+$ cells and diminished neurologic function over a study period of 1 year.

Methods

This study was approved by the institutional review board of the National Cardiovascular Center. All subjects provided written informed consent. A total of

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40 individuals with history of cerebral infarction (3 years or more from the last onset of stroke) were enrolled and followed for 1 year. Exclusion criteria included the following: patients who experienced a vascular event within 30 days of enrollment, patients with neurodegenerative diseases including Alzheimer's-type cognitive impairment, history of cerebral hemorrhage, cerebral infarction not classified according major causes (lacunar, atherothrombotic, or cardiogenic embolism), evidence of infection, malignant disease, and/or premenopausal women. On the day the first blood sample was obtained and 1 year after, all individuals were evaluated using the National Institutes of Health Stroke Scale (NIHSS), modified Rankin Scale (mRS), Barthel Index (BI), and Clinical Dementia Rating (CDR) by a single examiner masked to the experimental protocol and level of circulating $CD34^+$ cells. Hypertension, hyperlipidemia, and diabetes mellitus were defined based on the need for oral anti-hypertensive, anti-hyperlipidemic, or oral anti-diabetic drug therapy (or insulin), respectively, prescribed by the primary care physician. Smoking was defined as a history of >2 years and/or smoking in the last year. Using a modification of the International Society of Hematotherapy and Graft Engineering (ISHAGE) Guidelines (Sutherland *et al*, 1996), the number of circulating $CD34^+$ cells was quantified as described (Kikuchi-Taura *et al*, 2006) at the point of the entry and 1 year later. In brief, blood samples were incubated with phycoerythrin (PE)-labeled anti-CD34 antibody, fluorescein isothiocyanate (FITC)-labeled anti-CD45 antibody, 7-aminoactinomycin-D (7-AAD), and internal control (all of these reagents are in the Stem-Kit, BeckmanCoulter, Marseille, France). 7-AAD-positive dead cells and CD45-negative cells were excluded, and the number of cells forming a cluster characteristic of $CD34^+$ cells (i.e., low side scatter and low-to-intermediate CD45 staining) was counted. The absolute number of $CD34^+$ cells was calculated using the internal control. On the basis of our previous studies, the cumulative intraassay coefficient of variation of the measurement was 7.4% and test–retest intraclass correlation of the level of $CD34^+$ cells is 0.88 (Taguchi *et al*, 2004*a*). For statistical analysis, JMP version 5.1J was used. Individual comparisons were performed using a Mann–Whitney's *U*-test, χ^2-test, or two-tailed unpaired Student's *t*-test. Pearson's correlation coefficient was used to evaluate the correlation of the levels of $CD34^+$ cells between measurements. Mean ± s.e. is shown.

Results

To investigate the possible relationship between circulating $CD34^+$ cells and changes in neurologic status over the 1-year-study period, individuals were divided into two groups according to the level of circulating $CD34^+$ cells at the point of the entry. Baseline characteristics of the

Table 1 Baseline characteristic

	Total	*Group low*	*Group high*	P-*value for trend*
N	40	20	20	
At the point of entry				
No. of $CD34^+$ cells (per μL)	0.65 ± 0.07	0.34 ± 0.03	0.93 ± 0.10	
Age (years)	73.1 ± 1.1	72.9 ± 1.4	73.4 ± 1.7	0.85
Male gender, *n* (%)	28 (70)	12 (60)	16 (80)	0.16
Time from last stroke (years)	4.5 ± 0.2	4.5 ± 0.3	4.6 ± 0.3	0.75
Etiology, n *(%)*				0.83
Lacuna	25 (63)	13 (65)	12 (60)	
Atherothrombotic	12 (30)	6 (30)	6 (30)	
Cardiogenic embolism	3 (8)	1 (5)	2 (10)	
Risk factor, n *(%)*				
Hypertension	24 (60)	12 (60)	12 (60)	1.00
Hyperlipidemia	15 (38)	8 (40)	7 (35)	0.74
Diabetes mellitus	6 (15)	4 (20)	2 (10)	0.37
Smoking	8 (20)	5 (25)	3 (15)	0.42
Other cardiovascular disease	9 (23)	3 (15)	6 (30)	0.26
Treatment, n *(%)*				
Ca-channel blockers	13 (33)	7 (35)	6 (30)	0.74
ARB	14 (35)	7 (35)	7 (35)	1.00
ACE inhibitor	3 (8)	2 (10)	1 (5)	0.54
Diuretic	2 (5)	1 (5)	1 (5)	1.00
Beta-blockers	0 (0)	0 (0)	0 (0)	NA
Aspirin	19 (48)	7 (35)	12 (60)	0.11
Ticlopidine	8 (20)	6 (30)	2 (10)	0.11
Statin	14 (35)	8 (40)	6 (30)	0.51
One year after				
No. of $CD34^+$ cells (per μL)	0.69 ± 0.07	0.42 ± 0.05	0.97 ± 0.09	<0.001

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; NA, not available.

groups are shown in Table 1. Comparing these groups, there were no significant differences in age, gender, etiology of cerebral infraction, hypertension, hyperlipidemia, diabetes mellitus, smoking, and drug treatments. In univariate analysis, each cerebrovascular risk factor, including hypertension ($P=0.46$), hyperlipidemia ($P=0.35$), diabetes mellitus ($P=0.12$), and smoking ($P=0.35$), was not significantly correlated with a decrease in the number of circulating CD34+ cells. Treatment with a Ca-channel blocker ($P=0.73$), angiotensin-converting enzyme (ACE) inhibitor ($P=0.053$), angiotensin II receptor blocker (ARB) ($P=0.53$), diuretics ($P=0.52$), statins ($P=0.47$), aspirin ($P=0.86$), and/or ticlopidine ($P=0.80$) also did not correlate with a consistent difference in the number of circulating CD34+ cells. Each cerebrovascular risk factor and particular drug treatment was also not associated with a significant difference in neurologic function in 1 year, based on NIHSS, mRS, BI, and CDR (data not shown). At the point of entry, there were no significant differences in neurologic or cognitive function between groups (Figures 1A–1D). Compared with levels of circulating CD34+ cells in non-stroke control subjects presented in our previous report (0.81 ± 0.06 cells/μL; age, 74.2 ± 0.7; $n=32$) (Taguchi *et al*, 2008), the level of circulating CD34+ cells was significantly reduced in patients in the CD34+ cell low group in the current study ($P<0.001$). There was no significant difference between the level of circulating CD34+ cells in the CD34+ cell high group (in the current study) and the previously reported value ($P=0.20$; Taguchi *et al*, 2008). During the period of our observation, no patients had special exercise training, other than intensive rehabilitation in patients who had recurrent strokes.

During the 12-month-study period, 5 patients had recurrent strokes (3 patients in the lower CD34+ and 2 in the higher CD34+ group, respectively; $P=0.63$ between groups). After 12 months, neurologic and cognitive functions of all patients were reexamined, and changes in each score were recorded. Although there was no significant difference in the NIHSS score between groups (Figure 1E, $P=0.28$), there was significant worsening in neurologic function, based on BI in patients with decreased levels of CD34+ cells versus the group with increased levels (Figure 1F, $P=0.04$). Similarly, a trend towards worsening of mRS occurred in patients with decreased levels of CD34+ cells versus the group with increased levels, although these results did not achieve statistical significance (Figure 1G, $P=0.65$). In terms of cognitive function, a significant worsening in the CDR score was observed in patients with decreased levels of CD34+ cells, compared with the higher CD34+ cell group (Figure 1H, $P=0.002$). It is notable that no individual in the highest quartile ($n=10$) for levels of CD34+ cells displayed worsening of the CDR or BI score over the 1-year-study period. In the analysis of the patients without a recurrent stroke, a similar trend was observed (Figures 1I–1L), although the change of BI did not achieve statistically significant ($P=0.08$). Analysis of the correlation coefficient of the levels of CD34+ cells between at the point of the entry and 1 year later revealed significant strong correlation in patients without recurrence ($P<0.001$, $R^2=0.68$).

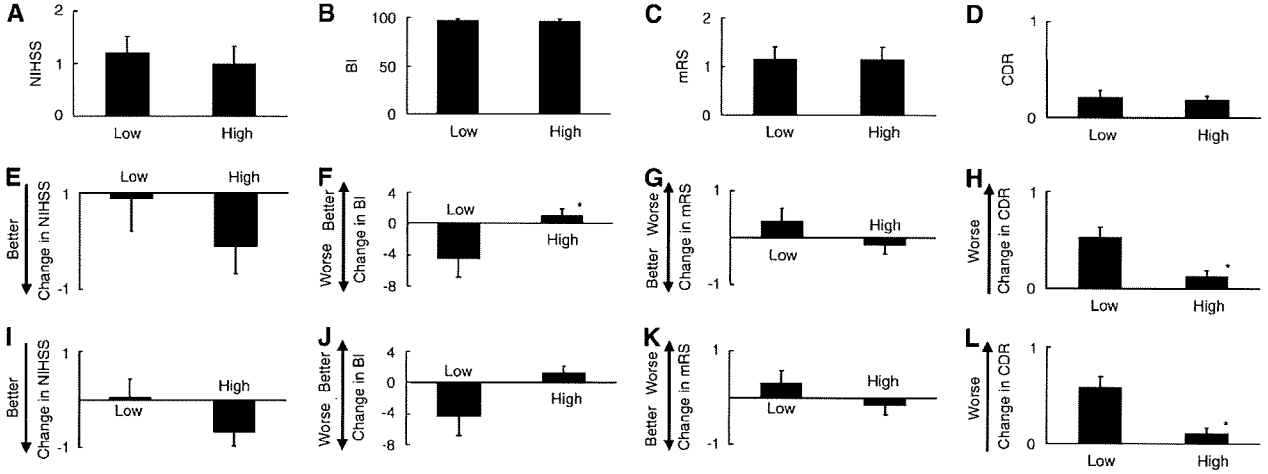

Figure 1 The level of circulating CD34+ cells and neurologic function in the study group after 1 year. (**A–D**) At the point of entry, there were no significant differences in the level of neurologic function, including NIHSS (**A**), BI (**B**), mRS (**C**), and CDR (**D**). (**E–H**) There was a trend suggesting accelerated worsening of neurologic function, evaluated by NIHSS, in patients with decreased levels of circulating CD34+ cells, although this did not achieve statistical significance (**E**). Compared with BI scores in patients with increased levels of circulating CD34+ cells, significant worsening was observed in patients with decreased levels of CD34+ cells (**F**). There was a trend of worsening of mRS in patients with decreased levels of circulating CD34+ cells, although this did not achieve statistical significance (**G**). Significantly poorer CDR scores were observed in patients with decreased levels of CD34+ cells, compared with those with increased levels of CD34+ cells (**H**). (**I–L**) Analysis of patients without recurrent strokes showed nonsignificant differences, but a similar trend was observed in changes in NIHSS (**I**), BI (**J**), and mRS (**K**). Poorer CDR scores were observed in patients with decreased levels of CD34+ cells, compared with those with increased levels of CD34+ cells (**L**), and this difference achieved statistical significance. *$P<0.05$ versus patients with decreased levels of circulating CD34+ cells.

Discussion

In this study, we have found that the level of circulating CD34+ cells has prognostic value for neural function in support of activities of daily living (BI) and cognitive function (CDR) in patients with a history of cerebral infarction. This result is potentially consistent with a role of CD34+ cells in maintenance of cerebral vasculature.

Similar to the correlation between mobilization of CD34+ cells and improved myocardial function after a coronary ischemic event (Wojakowski *et al*, 2006), mobilization of circulating CD34+ cells has been shown to correlate with functional recovery during the acute phase of cerebral infarction (Dunac *et al*, 2007; Yip *et al*, 2008). Our report herein shows a relationship between increased levels of CD34+ cells and improved functional outcome even in the extensive phase after stroke. These observations may reflect a close relationship between angiogenesis and neurogenesis under physiologic (Louissaint *et al*, 2002), as well as pathologic (Taguchi *et al*, 2004*b*) conditions.

The level of EPCs can be quantified using an assay for endothelial colony formation or fluorescence-activated cell-sorting analysis with multiple markers, including CD34 and kinase insert domain receptor (KDR) (Werner *et al*, 2005). Although the population of CD34+ cells is enriched in EPCs, it comprises multiple and heterogeneous subpopulations, indicating the possible advantage of selectively quantifying EPCs. However, measurement of EPCs is quite inexact, as large variations in their levels have been reported (i.e., by ~100-fold between reports) (Fadini *et al*, 2006*a*; Werner *et al*, 2005). Thus, there appears to be a need to standardize measurement of EPCs, in addition to a requirement for a relatively large blood volume to do the assay (for example, Loomans *et al* collected a 60 mL blood sample for EPC analysis) (Loomans *et al*, 2004). Our method for quantification of CD34+ cells is simple, reproducible (Kikuchi-Taura *et al*, 2006) and requires only 200 μL of peripheral blood. The latter method is suitable for screening a broad group of patients at risk for cerebrovascular disorders. Furthermore, CD34+ cells have been shown to secrete multiple growth/angiogenesis factors (Majka *et al*, 2001), contributing to maintenance of the microvasculature in addition to serving as a source of EPCs. These considerations indicate the value of quantitating peripheral CD34+ cells as a clinical biomarker in patients with vascular disease, not only as a substitute for quantifying EPCs.

In conclusion, our results indicate that circulating CD34+ cells in patients with cerebral ischemia have a positive impact on the course of disease, in terms of maintenance of neurologic function. In contrast, decreased levels of circulating CD34+ cells, possibly because of 'exhaustion' of the bone marrow or inability to mount an increase in cell counts, are associated with deterioration of neurologic status. Taken together with our previous results indicating that the level of circulating CD34+ cells can be correlated with cerebral blood flow and cerebral metabolic rate in patients with chronic cerebral hypoperfusion (Taguchi *et al*, 2004*a*), our present findings provide further support for a contribution of circulating CD34+ cells in maintenance of neurologic function in settings of ischemic stress. Although further basic and clinical studies will be required, we speculate that treatments with the goal of increasing levels of circulating CD34+ cells have the possibility of improving neurologic outcome in patients with impaired cerebral microcirculation.

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Conflict of interest

We declare that we have no conflicts of interest.

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Frequent Premature Atrial Contractions in Stroke of Undetermined Etiology

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Key Words
Cerebral infarction · Ischemic stroke · Premature atrial contraction · Paroxysmal atrial fibrillation

Abstract
Background: The cause of ischemic stroke is undetermined in 15–40% of patients. We studied the association between frequent premature atrial contractions (PAC) and stroke of undetermined etiology. ***Methods:*** We retrospectively studied ischemic stroke patients who were consecutively admitted to our department and underwent 24-hour Holter ECG recording including 163 noncardioembolic stroke patients (group A), 24 stroke patients of undetermined etiology (group B), and 37 cardioembolic stroke patients with prior-diagnosed paroxysmal atrial fibrillation (group C). The number of PAC per 24 h and the prevalence of patients with frequent PAC (200 or more per 24 h) were compared between the groups. ***Results:*** The number of PAC was significantly larger in groups B and C than in group A. The proportion of patients with frequent PAC was larger in group B (13/24, 54%) and in group C (18/37, 48%) than in group A (32/163, 20%). ***Conclusion:*** Our data suggest that frequent PAC should be regarded as a masked type of paroxysmal atrial fibrillation and should be included in one of the causes of cardioembolic stroke.

The cause of ischemic stroke is undetermined in 15–40% of patients [1, 2]. Paroxysmal atrial fibrillation (PAF) should be one of the latent causes of stroke of undetermined cause, since even long-term ECG recording may be unable to catch a potential episode of intermittent atrial fibrillation (AF) [3]. Patients with AF develop stroke approximately five times more frequently as compared with those without AF [4]. Patients with PAF have stroke rates similar to those of patients with sustained AF [5]. Therefore, it is important to predict the risk of developing PAF in patients with stroke of unknown etiology who have sinus rhythm.

AF is known to be associated with frequent premature atrial contractions (PAC) [6, 7]. We hypothesized that a certain population with stroke of unknown etiology should have latent PAF and such patients should be associated with frequent PAC. Engstrom et al. [8] reported that the risk of ischemic and hemorrhagic stroke in men is associated with a high frequency of atrial ectopic beats. Wallmannn et al. [9] reported that a high frequency of atrial premature beats in ischemic stroke patients predicts PAF. However, the association between frequent PAC and ischemic stroke of unknown etiology has not yet been studied. In this investigation, we studied the association between frequent PAC and stroke of unknown etiology.

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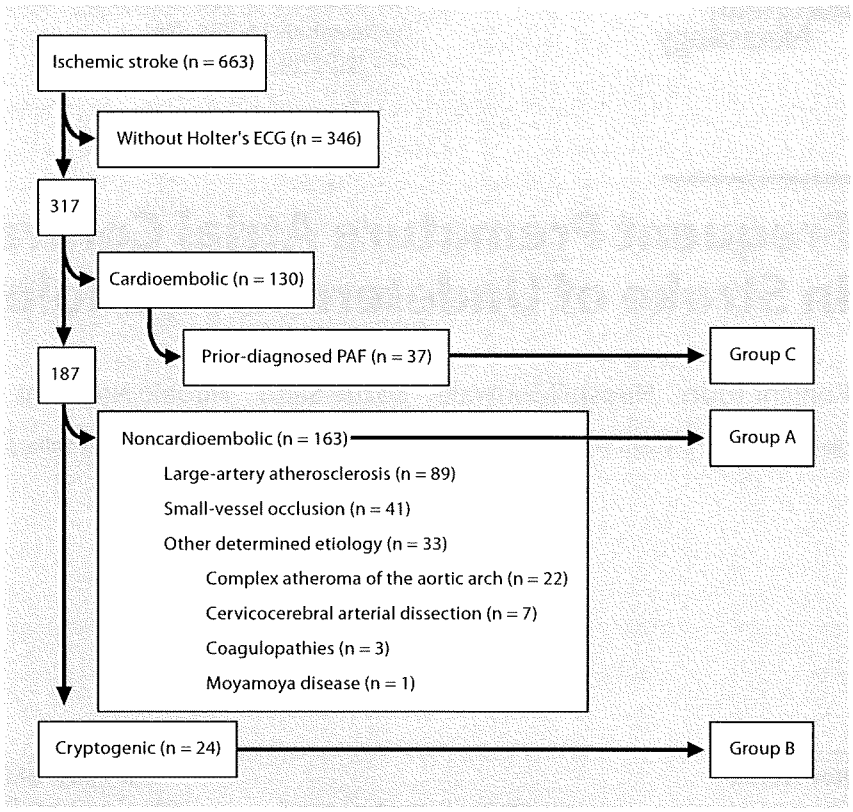

Fig. 1. Inclusion criteria for this study. Among the 317 ischemic stroke patients with Holter ECG recording, we included 163 noncardioembolic stroke patients (group A), 24 cryptogenic stroke patients (group B), and 37 cardoembolic stroke patients with prior-diagnosed PAF (group C).

Methods

We retrospectively studied ischemic stroke patients who were consecutively admitted to our department and underwent 24-hour Holter ECG recordings. Among the 663 ischemic stroke patients, we excluded 346 patients without Holter ECG recording. Among the 317 patients with Holter ECG recording, 89 patients had large-artery atherosclerosis, 41 patients had small-vessel occlusion, 33 had stroke of other determined etiology, 24 had stroke of undetermined etiology, and 130 had cardioembolic stroke as diagnosed according to the TOAST [10] classification criteria. Among the 130 patients with cardioembolic stroke, 37 patients had a history of PAF, but their Holter ECG in this study failed to detect AF. We excluded cardioembolic stroke patients but included these 37 patients with prior-diagnosed PAF as positive controls. We classified these patients into three groups: group A with 163 noncardioembolic stroke patients, including 89 cases of large-artery atherosclerosis, 41 cases of small-vessel occlusion, and 33 cases of other determined etiology; group B with 24 stroke patients of undetermined etiology, and group C with 37 cardioembolic stroke patients with a prior history of PAF (fig. 1). The number of PAC per 24 h and the prevalence of patients with frequent PAC were compared between the groups.

In group A, large-artery atherosclerosis was defined as the presence of at least 50% of stenosis or occlusion of the corresponding vessel, and small-vessel occlusion was defined as a small and deep infarct less than 15 mm in diameter in patients with hypertension. Complex atheroma of the aortic arch was defined as plaque thickening of at least 4.0 mm evaluated with transesophageal echocardiography. Cervico-cerebral arterial dissection was defined as the presence of characteristic angiographic findings such as double lumen, intimal flap, pearl and string sign, tapered narrowing or occlusion, retention of contrast medium, string sign, and aneurysmal outpouching. Coagulopathies included protein C or protein S deficiency. Cardioembolic cause included AF, recent myocardial infarction, akinetic left ventricular segment, dilated cardiomyopathy, patent foramen ovale, and left atrial or left ventricular thrombus or tumor. Stroke of undetermined etiology was defined as the absence of a definite cause after a standard workup including routine blood tests and a coagulation study (including tests for protein C, protein S, antithrombin III, and antiphospholipid antibody), Holter ECG, transesophageal echocardiography, transthoracic echocardiography, carotid ultrasonography, intracranial magnetic resonance imaging, and intracranial magnetic resonance angiography. We used a tape recorder with bipolar electrodes in the V2 and V6 positions for ECG recording. A reduced RR interval of 25% or more, presence of a P-wave, and a QRS width of 0.12 s or less were used as PAC criteria. Frequent PAC were defined as the fifth quintile of group A, i.e., 200 PAC or more per 24 h.

Age, sex, risk factors for stroke and NIHSS score on admission were recorded for each patient. The risk factors considered in this

Table 1. Baseline clinical characteristics and proportion of frequent PAC

	Group A (n = 163)	Group B (n = 24)	Group C (n = 37)	p value		
				A vs. B	B vs. C	A vs. C
Age (mean ± SD), years	66.3 ± 11.9	67.9 ± 8.4	69.7 ± 11.9	NS	NS	NS
Male/female ratio	116/47	15/9	25/12	NS	NS	NS
Hypertension	126 (77)	13 (54)	20 (54)	0.032	NS	0.0076
Diabetes mellitus	114 (70)	4 (17)	6 (16)	<0.0001	NS	<0.0001
Hyperlipidemia	96 (59)	8 (33)	9 (24)	0.033	NS	<0.001
Smoking	103 (63)	7 (29)	6 (16)	0.0033	NS	<0.0001
NIHSS score (mean ± SD)	5.6 ± 5.8	6.2 ± 5.7	8.9 ± 5.7	NS	NS	0.02
Frequent PAC	32 (20)	13 (54)	18 (48)	<0.001	NS	<0.001

Frequent PAC are defined as 200 PAC or more per 24 h. Figures in parentheses are percentages.

study were hypertension (casual blood pressure 140/90 mm Hg or more, or on medication), diabetes mellitus (fasting plasma glucose 7.77 mmol/l or more, glycosylated hemoglobin 6.2% or more, or on medication), hyperlipidemia (serum total cholesterol 5.70 mmol/l or more, or on medication), and smoking. Patients were categorized as smokers if they smoked at least one cigarette per day and as nonsmokers if they had never smoked or had stopped smoking 3 years previously.

Continuous data are expressed as mean values ± SD. The Mann-Whitney U test, Student's t test, and Pearson's χ^2 test were used for comparison of groups. Statistical significance was set at $p < 0.05$.

Results

The number of PAC was significantly larger in groups B and C than in group A (fig. 2). The proportion of patients with frequent PAC was higher in group B (13/24, 54%) and in group C (18/37, 48%) than in group A (32/163, 20%) (table 1). In group A, the number of patients with frequent PAC was 21 of 89 with large-artery atherosclerosis, 5 of 41 with small-vessel occlusion, and 6 of 33 with other determined etiology. However, there was no significant difference among these subgroups.

Baseline clinical characteristics are summarized in table 1. Mean age and proportion of male sex did not differ between the three groups. Group A patients had hypertension, diabetes mellitus, hyperlipidemia, and current smoking habit, which are known to be associated with large-artery atherosclerosis, and small-vessel occlusion, more frequently than group B and C patients. The NIHSS score was higher in group C than in group A.

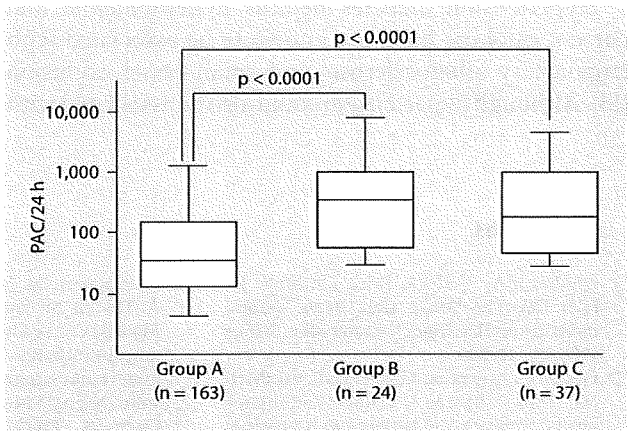

Fig. 2. The number of PAC per 24 h. Box plots indicate 10th, 25th, 50th, 75th, and 90th percentiles of noncardioembolic stroke patients (group A), cryptogenic stroke patients (group B), and cardoembolic stroke patients with prior-diagnosed PAF (group C). The number of PAC was significantly larger in groups B and C than in group A.

Discussion

Frequent PAC is known to be associated with PAF [6, 7]. After coronary artery bypass grafting and aortic valve replacement, frequent PAC can predict AF [11, 12]. Wallmann et al. [9] have reported that frequent PAC can be a surrogate marker for PAF in stroke patients without AF, including lacunar and atherothrombotic stroke patients. However, PAF in patients with lacunar or atherothrombotic stroke is not always the cause of stroke. To the best of our knowledge, this is the first study reporting the as-

sociation of frequent PAC with stroke of undetermined etiology. Our study found that frequent PAC was associated with stroke of undetermined etiology and with cardioembolic stroke with prior-diagnosed PAF. These findings suggest that frequent PAC in stroke of undetermined etiology should be regarded as a masked type of PAF and as one of the causes of cardioembolic stroke. Holter monitoring in ischemic stroke patients will identify new AF/flutter in approximately 1 in 20 patients. Extended duration of monitoring may improve the detection rate [13]. We suggest that repetitive Holter ECG recordings are needed to detect latent PAF at least in patients with stroke of undetermined etiology, especially with frequent PAC. A 72-hour recording time, compared to a 24-hour period, was reported to improve the detection of PAF in ischemic stroke patients [3].

Hypertension, diabetes mellitus, hyperlipidemia, and current smoking habit are known to be associated with large-artery atherosclerosis and small-vessel occlusion [14]. Although hypertension could also be associated with frequent PAC, group B and C patients had hypertension less frequently than group A patients. A Severe neurologic symptom is known to be associated with cardioembolic stroke [14]. In this study, the NIHSS score is significantly higher in group C than in group A, but not in group B.

The limitation of the present investigation is that the study was performed in a retrospective manner. There is potential bias because we did not define the indication criteria for Holter ECG recording in this retrospective study. However, we showed that the presence of frequent PAC was more commonly associated with stroke of undetermined etiology than with noncardioembolic stroke. We also showed that stroke of undetermined etiology and cardioembolic stroke with a prior history of PAF were similarly associated with frequent PAC. These results of the present study confirm the findings of previous studies showing an association between frequent PAC and stroke [8, 9]. Further prospective studies are needed to clarify the risk of stroke in patients with frequent PAC.

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知って得する最新情報

脳血管障害に対する幹細胞治療法の開発

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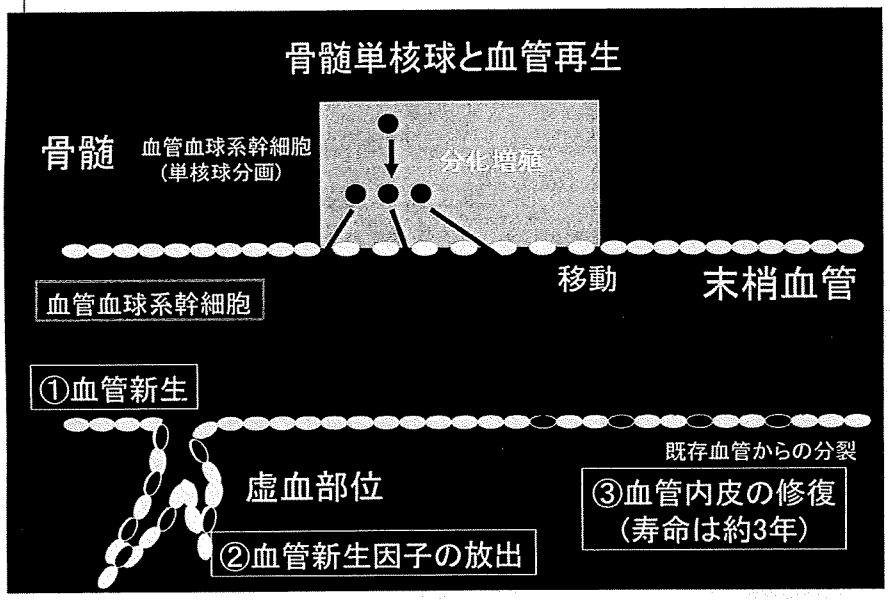
はじめに

現在わが国においては，急速な高齢化社会を迎えており，それに伴う要介護者の急激な増加は極めて深刻な社会問題である．特に要介護者発生原因の40%以上が脳血管障害など中枢神経障害であり，これらの疾患に対する有効な治療法の開発は緊急の課題である．中枢神経障害の治療を目指した神経幹細胞移植に関する研究は国内外の非常に多くの施設で精力的に行われているが，米国で行われた脳梗塞患者に対する胎生期脳由来神経幹細胞移植やヒト腫瘍細胞由来神経細胞移植治療に関する臨床治験では十分な治療効果が認められておらず，脳梗塞後の神経機能の改善には単なる神経幹細胞移植ではほとんど効果がないことが明らかにされつつある．

われわれは胎生期発生過程や成体 Song Bird 等における脳神経組織の再生において，神経新生・神経再生は血管新生と平行してプログラムされていることに注目し，中枢神経障害後の血管再生と神経再生の関連について精力的な研究を行った結果，脳血管再生により誘導された再生神経が脳神経機能の著明な改善をもたらすことを世界に先駆けて明らかにする[1]とともに，これらの知見を治療法として発展させるための研究を行ってきた．本稿では，脳血管障害患者に対する自己骨髄単核球を用いた幹細胞治療に関するわれわれの取り組み，およびその将来展望に関して概説する．

脳梗塞後における血管再生と神経再生

虚血性循環器疾患に対する幹細胞を用いた臨床試験においては，心筋梗塞患者や四肢虚血患者などにおいて自己骨髄単核球移植の治療効果が明らかにされてきた[2]．骨髄には図に示すように血管血球系幹細胞が存在するとともに，末梢血中への移動後，虚血領域において血管内皮細胞への分化および血管新生因子の分泌を行うことが明らかにされている．臨床試験では，これらの血管再生作用の強い細胞群が含まれる単核球分画が移植されたことにより，虚血領域における治療効果が得られたと考えられている．



これらの知見を脳血管障害患者に応用するため，われわれは再現性の非常に高い脳梗塞モデルを確立し，骨髄系血管血球系幹細胞の脳梗塞に対する治療効果の検討を行った[1]．その結果，① 脳梗塞後の骨髄単核球移植など血管血球系幹細胞投与は血管再生を促進すること，② 脳梗塞後の血管再生は内因性の神経再生を促進すること，③ 脳梗塞後の血管再生が内因性神経幹細胞の生着に必須であること，④ 血管血球系幹細胞投与による脳梗塞後の血管再生は脳神経組織の再生を誘導すること，⑤ 脳梗塞後の血管再生による脳組織再生は脳機能の再生をもたらすこと，更に，⑥ 血管血球系幹細胞を骨髄から動員する作用のある G-CSF の投与は逆に脳萎縮や神経機能が低下すること[3]，

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などを示してきた．また，細胞投与時期や必要細胞数などの検討を行い，脳梗塞後2，4，7，10日および14日後の投与において脳梗塞巣の減少，および行動の改善を認めるものの，脳梗塞後1日および21日後においては有意な再生促進作用がないことを明らかにしている．

次に，われわれは脳梗塞患者に対する自己骨髄単核球移植に関する前臨床試験として，霊長類における脳梗塞後の骨髄採取およびその静脈内投与に関する検討を行った．投与骨髄単核球の体内分布の検討においては，頭部に集積がみられるとともに脾臓に多くの集積がみられ，肺にはほとんど集積がみられなかった．骨髄単核球投与前後における末梢血中血管血球系幹細胞の定量的評価では，細胞投与1時間後においても末梢血中CD 34陽性細胞数の約2.5倍の増加が観察された．また脳梗塞後の経時的神経学的評価においては，細胞投与後に順調な機能回復が観察された．

さらに，われわれは脳梗塞患者における神経再生に関する病理的検討を行い，血管再生および神経再生のピークはそれぞれ脳梗塞数日後，および2〜3週間後であり，マウスにおける脳梗塞後の再生過程と類似していることを明らかにしている．これらの所見は，ヒトにおいても脳梗塞急性期〜亜急性期において内因性神経幹細胞誘導が起こることを示しており，ヒトにおける血管再生療法の可能性を示す重要な所見であると考えている．

心原性脳塞栓症患者に対する自己骨髄単核球移植による臨床試験

以上の知見に基づき，平成19年10月「厚生労働省ヒト幹細胞を用いる臨床研究に関する指針」による認可を経て，国立循環器病センターでは心原性脳塞栓症患者に対する自己骨髄単核球移植による臨床試験のエントリー患者の募集を開始している．この臨床試験では，対象患者群を重症心原性脳塞栓患者(NIHSS 10点以上かつ入院時からの回復が5点以下)とし，脳梗塞発症7〜10日後に局所麻酔下において自己骨髄細胞を採取し，骨髄単核球分画を精製後に静脈内への投与を行う．一次エンドポイントは，① 投与1ヵ月後のNIHSSの改善度(有効性)，② NIHSS悪化症例の頻度(安全性)である．予定症例数は低用量群(骨髄採取25 m*l*)6名と高用量群(骨髄採取50 m*l*)6名であり，過去に国立循環器病センターに重度心原性脳塞栓症で入院した患者群をコントロールとして用いる予定である．

脳血管障害に対する細胞治療の展望

脳梗塞においては，神経細胞だけでなく脳脊髄関門を形成する細胞群などが障害されるため，脳の組織修復および脳神経機能の改善にはこれらの脳血管や支持細胞群を含む神経組織全体の再構築が望まれている．われわれは，脳梗塞後の機能再生には血管再生を中心とした内因性組織修復機構の促進が非常に重要であることを示してきたが，内因性組織修復機構の促進を基盤とした神経幹細胞移植，さらには移植神経幹細胞の分化制御や機能制御を行うことにより，さらに有効な治療法として発展させていくことができると考えている．

また，脳血管障害・脳血管性認知症の予防に関しては，脳血管障害患者における末梢血中血管血球系幹細胞の減少が，① 脳循環代謝の悪化，② 認知機能異常，③ 認知機能の経時的な低下と関連していること，および，④ 糖尿病患者においては末梢血中血管血球系幹細胞の減少が糖尿病性心筋障害と関連している，など末梢血中血管血球系幹細胞と微小循環障害・再生機転に関する非常に重要な様々な知見を得ている[4〜6]．血管血球系幹細胞を用いた治療法は急性虚血性疾患患者に対する有効な治療法となるだけでなく，脳血管性認知症や糖尿病性腎症微小循環障害，糖尿病性心不全患者など，生活習慣病などに伴う幅広い疾病群に対する根治的・普遍的な予防法として発展可能であると考えており，その予防法確立に向けた研究を現在推進中である．

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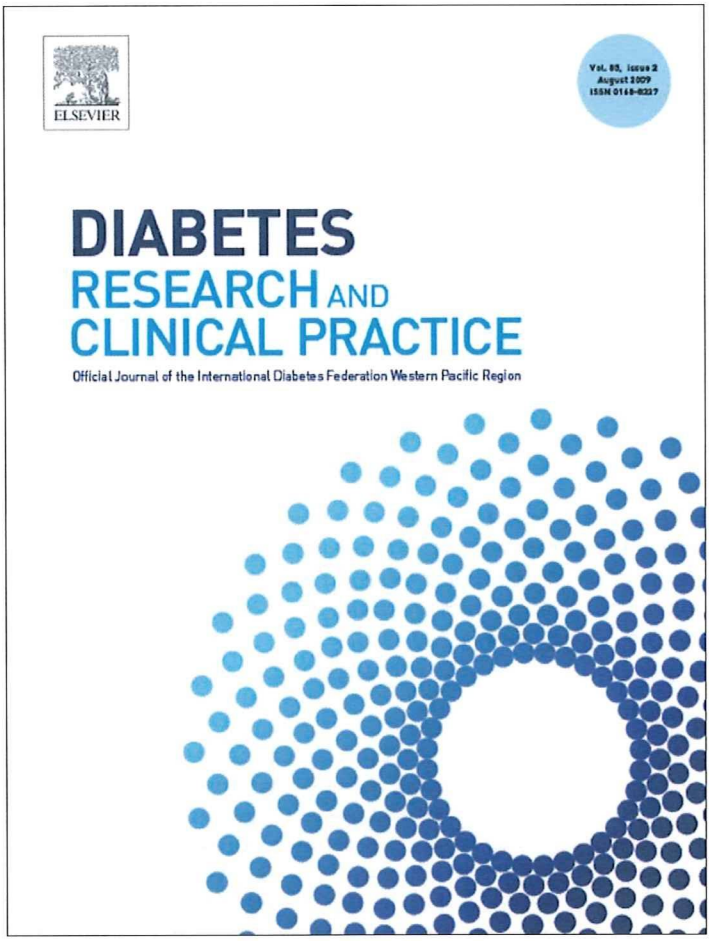

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Association study of 11β-hydroxysteroid dehydrogenase type 1 gene polymorphisms and metabolic syndrome in urban Japanese cohort

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ABSTRACT

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), one of the isoforms of the 11β-hydroxysteroid dehydrogenase enzymes, acts as an oxo-reductase to reactivate cortisone to cortisol, plays a critical role in tissue-specific corticosteroid reactions, and is therefore a key molecule associated with the development of metabolic syndrome. We investigated whether variations in the 11β-HSD1 gene correlated with metabolic syndrome. We performed case–control study using a population-based urban Japanese cohort. Among 3005 urban residents, we examined 431 subjects diagnosed with metabolic syndrome according to the Japanese definition and 777 subjects with none of metabolic syndrome criteria as control. We genotyped three single nucleotide polymorphisms (SNPs) (+9410T>A, +17925C>T, +27447G>C) across the 11β-HSD1 gene in them and analyzed the associations of SNPs and haplotypes with metabolic syndrome. The +9410A allele showed a tendency to metabolic syndrome (OR = 1.5, 95%C.I., 1.0–2.2; P = 0.041 and Bonferroni corrected P = 0.123) without statistical significance. However, we could not find any significant association between metabolic syndrome and SNPs in the 11β-HSD1 gene. Our findings indicate that polymorphisms and haplotypes in the 11β-HSD1 gene are not significantly associated with metabolic syndrome in the Japanese population.

1. Introduction

Two isoforms of the 11β-hydroxysteroid dehydrogenase enzyme (11β-HSD), 11β-HSD type 1 (11β-HSD1) and 11β-HSD type 2 (11β-HSD2), catalyze the conversion between hormonally active cortisol and inactive cortisone [1]. 11β-HSD1 acts as an oxo-reductase that reactivates cortisone to cortisol [1] and is an abundant intracellular component in adipose tissue, liver and central nervous system [1–3]. In contrast, 11β-HSD2 is a dehydrogenase that inactivates cortisol to cortisone and is exclusively expressed in organs involved in water and electrolyte metabolism, such as the colon, kidney, sweat

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gland, and placenta [1,4]. This differential expression provides a mechanism for tissue-specific corticosteroid receptor activation that is independent of circulating cortisol concentrations [1,5]. Moreover, studies using animal models have shown that 11β-HSD1 increases intracellular glucocorticoid levels by converting circulating 11-dehydrocorticosterone (cortisone in humans) into active corticosterone (cortisol) through 11β-reductase in adipocytes decrease intracellular glucocorticoid levels [6–9]. In human, 11β-HSD activity in adipose tissue was positively correlated with BMI [10] and 11β-HSD1 inhibition enhances insulin sensitivity and provides a new approach to control metabolic diseases, including type 2 diabetes [11–13].

Epidemiologic studies have indicated that metabolic syndrome has become more prevalent in Western and Asian countries due to both environmental factors and lifestyle changes, such as a high-calorie diet and sedentary behavior. However, there is also evidence that certain individuals are genetically predisposed to metabolic syndrome and its related traits. Polymorphisms in the HSD11B1 gene which encodes 11β-HSD1 have been reported to be associated with type 2 diabetes [14] and hypertension [15]. In particular, Gelernter-Yaniv et al. reported the positive association of the ins4436A SNP in the HSD11B1 gene with BMI and insulin resistance in obese children [16]. However, this association has been inconsistent, probably because of differences in sample size and ethnicity [17].

In light of the possible involvement of 11β-HSD1 in metabolic syndrome, we investigated whether genetic variants of the HSD11B1 gene are associated with metabolic syndrome.

2. Methods

2.1. *Subjects and definition of metabolic syndrome*

We recruited 3655 residents on population-based cohort (Suita, Osaka Prefecture, Japan) from April 2002 to February 2004 and obtained written informed consent to study SNPs. The study design was approved by the Committee on Genetic Analysis and Gene Therapy and the ethics committee of the National Cardiovascular Center. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Of the 3655 participants, 3005 were included in the study because blood could be collected from them after a 12-h fast and because all three single nucleotide polymorphisms (SNPs) of the HSD11B1 gene in these subjects were successfully genotyped. According to the Japanese consensus determined by eight scientific societies including the Japanese Society of Internal Medicine, metabolic syndrome is defined as central obesity (waist circumference ≥85 cm for men and ≥90 cm for women) plus any two of the following three factors: dyslipidemia (triglycerides >1.69 mmol/l (150 mg/dl) and/or high-density lipoprotein (HDL) cholesterol <1.03 mmol/l (40 mg/dl), or lipid-lowering therapy), hypertension (systolic blood pressure (SBP) ≥130 and/or diastolic blood pressure (DBP) ≥85 mmHg, or antihypertensive therapy), and fasting plasma glucose ≥6.11 mmol/l (110 mg/dl) or previously diagnosed type 2 diabetes [18]. Subjects with none of these metabolic syndrome criteria were defined as controls. Among 3005 persons, 431 persons met the metabolic syndrome criteria, 777 persons did not meet any one of the metabolic syndrome criteria, and 1797 persons who belonged neither to metabolic syndrome nor to controls were indicated as intermediate in Table 1. The Japanese criteria for metabolic syndrome differ from those of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), which is considered present when at least three of the five traits including an increased waist circumference, blood pressure elevation, low HDL cholesterol, high triglycerides, and hyperglycemia. As we thought whether a 11βHSD gene was involved in the crises of the metabolic syndrome with the pathology which made visceral fat accumulation a base, we used the Japanese criertia for metabolic syndrome.

2.2. *Clinical parameters*

Blood pressure was measured after at least 10 min of rest in the sitting position. The mean values of two SBP or DBP measurements obtained by a physician using a mercury sphygmomanometer (recorded >3 min apart) were used for analysis. After 12 h of fasting, blood samples were collected, and total cholesterol, HDL-cholesterol, and triglyceride levels were measured with an autoanalyzer (Toshiba TBA-80) in accordance with the Lipid Standardization Program of the US Centers for Disease Control and Prevention through the Osaka Medical Center for Health Science and Promotion, Japan.

2.3. *Anthropometric estimates*

The participants, wearing no shoes and only underwear, were weighed on an electronic scale, and results were recorded to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using height meter with the subject standing. Waist diameters were measured to the nearest 1.0 cm at the height of the navel upon breath intake using a non-extendable linen tape measure.

2.4. *Screening and identification of SNPs in the human HSD11B1 gene*

Genomic DNA samples were isolated from peripheral leukocytes of the participants. Eight primer sets were designed to amplify the promoter and intron/exon boundaries of the HSD11B1 gene, and an initial SNP screening was performed using 48 randomly chosen DNA samples. Screening for genetic variants was performed using a denaturing HPLC method, in which the PCR products were analyzed using WAVE DNA Fragment Analysis and WAVEMAKER software 4.0 (Transgenomic Inc., Omaha, NE, USA), following the manufacturer's protocol. All detected variations were confirmed by a direct sequencing using an ABI 3700 (Applied Biosystems, Foster City, CA, USA). SNPs were genotyped using TaqMan PCR (ABI PRISM 7900HT, Applied Biosystems). The validity of the detection systems was verified prior to the large-scale study, using 48 samples that were genotyped at the initial screening.