■表3　高齢者糖尿病において評価すべきこと

・糖尿病の状態

病型、罹病期間、インスリン分泌・感受性、血糖コントロール、肥満、高血圧、脂質異常、血管合併症など

・他疾患の合併

他疾患の有無（心疾患、骨・関節疾患、悪性新生物など）、生命予後

・視力・聴力・尿失禁・コミュニケーション

・日常生活動作

基本的 ADL　　手段的 ADL

・精神・神経機能

認知機能（改訂長谷川式簡易知能検査、ミニメンタルテストなどで評価）うつ状態（Geriatric depression scale: GDS15 などで評価）意欲（鳥羽式スケールなどで評価）

・社会・経済的状況

家族構成とキーパーソン、住居、経済状況、介護保険の利用

④ 専門医紹介のポイント

◉糖尿病の急性発症または血糖コントロール増悪時

インスリン療法の導入は原則的に入院とする。体重減少を伴う例では、悪性腫瘍を合併することが少なくない。薬剤性、糖尿病以外の原因についても検査する。

◉急性代謝失調（高血糖高浸透圧症候群・糖尿病性ケトアシドーシス、遷延性低血糖、シックデイ）

◉血管合併症の検査（脳、心、下肢動脈、腎、眼、末梢神経障害など）

血管合併症の急性発症時には直ちに該当科での治療を要する。高齢者糖尿病では動脈硬化性疾患の合併頻度が高く、無症候性の脳梗塞、心筋虚血が多いことから、全身の血管障害の検査を積極的に行う。

◉高齢者に特有の合併症（認知症、うつ、骨病変・転倒、尿失禁など）

合併症、生活機能障害は、自立した療養を阻害する（図1）。とくに後期高齢者では、認知症の早期発見、転倒・骨折の予防が重要である。

（櫻井　孝）

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Loss of CO_2-induced Distensibility in Cerebral Arteries with Chronic Hypertension or Vasospasm after Subarachnoid Hemorrhage

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Abbreviations: ICA, internal carotid artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; ECA, external carotid artery; CCA, common carotid artery; MAP, mean arterial pressure; PCA, posterior cerebral artery; BA, basilar artery

We developed a rat cerebral angiography system using monochromatic synchrotron radiation X-rays at SPring-8, a third generation synchrotron radiation facility. Using new technique, we assessed the distensibility of major trunk arteries after subarachnoid hemorrhage (SAH) in normotensive and hypertensive rats. Twenty-five adult Wistar Kyoto rats (WKY) and fourteen stroke-prone spontaneously hypertensive rats (SHR) were prepared SAH by double hemorrhage injection method into cisterna magna. Angiography was performed on day 7 and was repeated three times in each rat before and after loading of hypercapnia at 100-120 mmHg of $PaCO_2$. The diameters of major trunk vessels were assessed. Light microscopic observation of artery lumen and wall were also performed. Angiographical vasospasm was demonstrated in basilar artery in WKY with 66 % reduction in diameter of control. In ICA and other major trunk in WKY and all the arteries in SHR did not demonstrate vasospasm. SHA resulted in loss of hypercapnia-induced distention in BA of WKY. In SHR, the distensibility was impaired regardless of hemorrhage. Histological study demonstrated basilar artery in WKY thickened at 184 % after SAH and became similar to non-hemorrhagic SHR. ICA in WKY and both BA and ICA in SHR were unchanged in wall thickness before and after SAH. High quality angiography demonstrated deteriorated distensibility in chronic hypertension or SAH-induced spastic vessels.

Subarachnoid hemorrhage (SAH) is often associated with a delayed vasospasm that is a major course of disability in patients after cerebral aneurysm rupture. Many researchers have used rodents to study the pathogenesis of cerebral vasospasm after SAH because small animals are less expensive and easy to handle. Rat models were developed in which blood was injected directly into the cisterna magna (3, 24). The degree of vasospasm is determined by measurement of the vessel diameters and direct observation through cranial

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bone window was used initially (2). Subsequently, angiography has been reported in rat SAH model, however, it is very difficult because of the small size of the animal. Furthermore, physiological change cannot be characterized as it has in models using other larger species.

Recently, we have developed a microangiography system using monochromatic synchrotron radiation X-rays at SPring-8, a third generation synchrotron radiation facility. With this system at SPring-8, high spatial resolution of 8 μm has been achieved using phantoms. We have firstly applied this system for cerebral microangiography in rats and mice (9, 13, 16, 25). In the present study, we have focused on in vivo assessment of spastic rat cerebral arteries after SAH. Impaired autoregulation of the cerebral blood flow has been known in rat SAH model between 2 and 5 days after SAH (20). This study was done with133-Xenon injection into carotid artery and reflected pathogenesis of small arteriole in the cortex. The major artery trunks, that are targets of interventional approach in patients, were not shown yet in previous studies. Using microangiography technique, we studied the change of distensibility of those major artery trunks by loading systemic hypercapnia.

Secondly, we studied the effects of chronic hypertension on vasospasm using spontaneously hypertensive rats (SHR). Chronic hypertension in SHR induces thick media in cerebral arteries in comparison with normotensive rats (15) and may affect the spastic change as well as distensibility in systemic hypercapnia. Although aging is known to increase the degree of vasospasm after SAH in rabbits, studies with chronic hypertension has not been done yet.

MATERIALS AND METHODS

Imaging System and Animal Preparation

All experimental procedures followed the guidelines for animal experimentations at Kobe University Graduate School of Medicine. The imaging was performed at the 2nd optical hatch of BL28B2 beamline at SPring-8 (Japan Synchrotron Radiation Research Institute) in Hyogo, Japan. Details of the imaging system used in the present study were, in part, previously described (13, 16).

Adult Wistar Kyoto rat (WKY, n=25) and adult SHR (n=14), weighting between 450 and 600g, aged 6 months, were used. The method to produce SAH is based on a double hemorrhage injection method. On day 0, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and allowed to breath spontaneously. Under sterile condition, rats were held in a 20° head down in a stereotaxic frame and the atlano-occipital membrane was exposed. A 27-gauge needle bent at the tip for a length of 2 mm was carefully inserted into the cisterna magna. On day 2, rats were anesthetized again and a second SAH was induced as described above. On day 7, imaging study was performed under pentobarbital sodium (50 mg/kg, i.p.) anesthesia, which has been reported in our previous reports (13, 16).

Experimental Protocol

After the animal preparation was completed, the PE-50 tube inserted into the ECA was connected to an automated injector (Nihon Koden, Japan) that was programmed to reproducibly deliver nonionic contrast media (Iomeprol) at 0.2 ml / 0.4 sec for ICA imaging and 0.5 ml / 1.0 sec for BA. This injection volume and speed were determined based on our pilot study, in which different volumes of the contrast media were injected at different injection rates to obtain the most acceptable and physiological images. Arterial blood gases were checked before the first imaging. The first angiogram was performed to estimate the basal tone of the vessels. We allowed at least 3-min intervals between angiograms to reestablish physiological blood flow before each angiographic study.

The inhalation of CO_2 at 12-15 % mixed in air was performed for 6 minutes. An angiogram, at the end of inhalation, was then performed. The arterial blood gases were analyzed and the inhalation was returned to normal air. An additional angiogram was performed at 10 min under normocapnia, and the arterial blood gases were analyzed. Rats were divided into four subgroups; a) WKY with SAH (n=17), b) WKY without SAH (n=5), c) SHR with SAH (n=9), d) SHR without SAH (n=8). Rats in group b) and d) were as control in which saline was injected into cisterna magnum instead of autologous blood.

Image Analysis

The images were stored digitally. The initial acquisition time for an image was 30 images per second. To make the subtraction images, ten original images were added and subtracted by the pre-infusion image. The vessel diameter was measured semiautomatically on the digital image with readymade software (Image ProPlus, U.S.A.) combined with a program developed for this study. A short temporary axis was delineated by hand at the vessels specified for measurement. The axis was delineated within 0.5 mm after branching for PCA, MCA and ACA. For ICA, the axis was in the middle of the segment between PCA and MCA. BA was measured at two points of one third proximal and one third distal. The density profile perpendicular to the temporary axis was calculated and distance between these two peaks of phase refractions of X-ray on the border of vessel wall was measured as a diameter of the vessel.

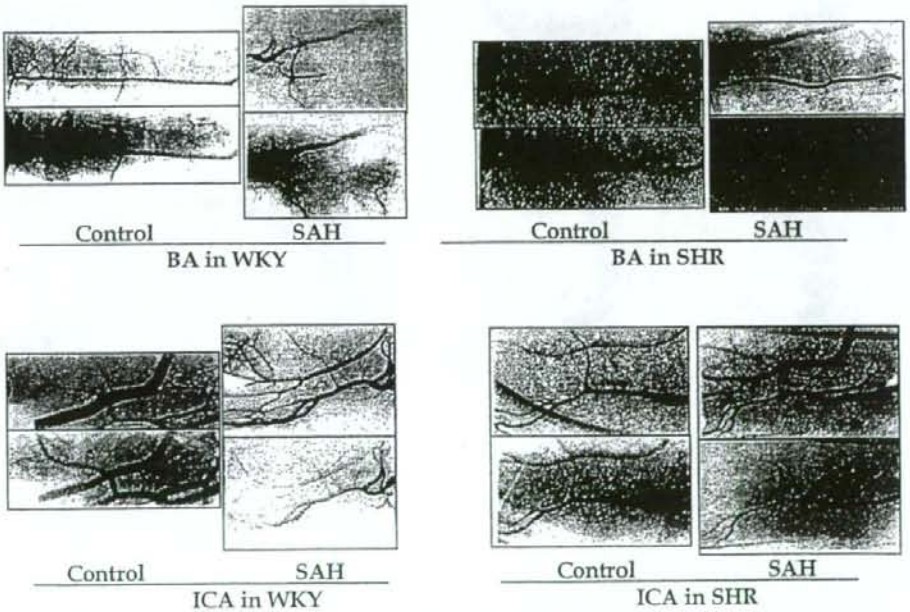

Figure 1: Representative images of rat cerebral vessels taken by SPring-8 and micorangiography technique. Uppper: normocapnia, lower: hypercapnia. BA: basilar artery, ICA: internal carotid artery, SHR: spontaneously hypertensive rat, WKY: Wistar Kyoto rat, SAH: subarachnoid hemorrhage.

Morphometric study

After angiography, rats were killed by overdose anesthesia and decapitated. Rats were perfused transcardially by 4% parahormaldehyde in phosphate buffer for 30 minutes. Brain were removed and soaked in 10 % parahormaldehyde for 7days. After paraffin embedding, slices containing basilar artery and internal carotid artery was made at the thickness of 10 μm and stained with hematoxylin and eosin. The internal diameter and wall thickness of the arteries were measured using a digitized image analysis system. The thickness was defined as the distance from the luminal surface to the outer border of the media at four different points.

Statistics

Data are presented as means +/- S.E.. An unpaired Student *t* test was used to detect significant differences when two groups were compared. Statistical differences among group means were determined by one-way ANOVA with repeated measures, followed by a post hoc comparison. A value of $P< 0.05$ was considered to be statistically significant.

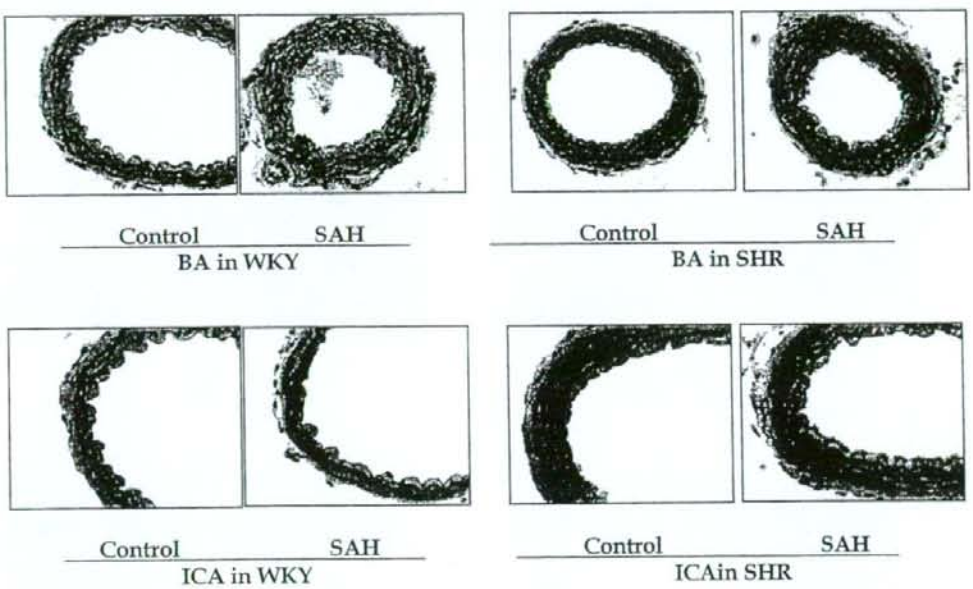

Figure 2: Histological demonstration of cross section in basilar arteries and internal carotid arteries.

RESULTS

With our experimental setting of 0.2 ml injection, the posterior fossa was not filled by the contrast media indicating selective ICA angiography was performed. Within the radiation field of 3 x 7.3 mm, the images of major trunk vessels of ICA from the entry point to intracranial space, PCA, MCA and ACA were consistently obtained. The posterior choroidal artery, the large branch of PCA was also within the field but it was always associated with overlapping images of thalamic branches in the axial view and this made analysis impossible. The image of posterior communication artery arising from the PCA was occasionally obtained by selective ICA angiography but mostly faint.

The vessel diameters of rats without SAH (SAH(-)) and rats with SAH(SAH(+)) measured by angiography were summarized in Table 1. In SAH(-) rats, the diameters of BA were significantly narrower in SHR than in WKY (132 μm vs.248 μm at proximal and 102 μm vs. 274 μm at distal; $p < 0.05$) and other major trunk vessels did not significantly differ between WKY and SHR. In SAH(+) rats, significant vasospasm was observed in BA of WKY (64-66 % of SAH(-)). Other major trunk vessels in SAH(+) WKY and all of the major trunk vessels in SAH(+) SHR-SP had no spastic changes (81-139 % of SAH(-)).

By loading CO_2, all of the trunk vessels in SAH(-) WKY demonstrated significant dilatation (125 –169 % of pre-CO_2 values for each) (Table 2). This dilatation ability was lost after SAH except PCA. In SHR, both SAH(-) and SAH(+) did not demonstrated vasodilatation by CO_2 loading at all.

The histological measurement of ICA and BA before SAH demonstrated significant narrower diameter in SHR compared with WKY (110 μm vs. 153 μm in BA and 218 μm vs. 297 μm in ICA) as shown in Table 3. SAH-induced narrowing of the vessels was not observed histologically in all. The measurement of arterial wall thickness demonstrated that in SAH(-) rats, both BA and ICA had significantly thicker wall in SHR compared with WKY (29.6 μm vs. 19.2 μm in BA and 33.3 μm vs. 29.2 μm in ICA). SAH-induced-pathological change was observed only in BA of WKY, in which arterial wall significantly thickened by SAH (181 % of SAH(-)). This thickened value of the diameter in BA of WKY was comparable to BA of SHR.

Table 1. Comparative measurments in WKY and SHR with subarachnoid hemorrhage

	vessel diameter (μm)			
	SAH(-)	SAH(+)	Spasm (%)	p Value
Basilar A.(proximal)				
WKY	248±21.3	164±34.7	66	0.02
SHR	132±33.1	149±32.3	112	NS
Basilar A.(distal)				
WKY	274±26.7	176±36.1	64	0.02
SHR	102±41.8	124±36.1	121	NS
ICA				
WKY	319±28.1	360±39.2	113	NS
SHR	340±27.7	306±37.5	99	NS
MCA				
WKY	214±30.3	235±21.5	109	NS
SHR	218±15.9	217±14.7	99	NS
ACA				
WKY	255±20.3	208±27.1	81	NS
SHR	226±18.6	226±21.2	100	NS
PCA				
WKY	180±28.5	251±15.0	139	NS
SHR	220±46.6	212±26.7	97	NS

Table 2. CO2 induced distention of trunk vessels in the chronic vasospasm

	WKY				SHR			
	SAH(-)		SAH (+)		SAH(-)		SAH (+)	
	mean±SE (%)	p value	mean±SE (%)	p value	mean±SE (%)	p value	mean±SE (%)	p value
Basilar A.(proximal)								
pre	238±21.3 (100)	P=0.04	164±34.7 (100)	NS	132±33.1 (100)	NS	148±32.3 (100)	NS
post	294±27.9 (125)		151±35.1 (99.7)		172±46.3 (130)		165±37.5 (11.5)	
rec/10min	236±37.7 (99.1)		145±51.6 (88.7)		155±48.2 (117)		152±46.6 (102)	
Basilar A.(distal)								
pre	268±26.7 (100)	P=0.03	176±36.1 (100)	NS	102±41.8 (100)	NS	124±36.1 (100)	NS
post	368±43.2 (137)		161±42.5 (91.1)		128±56.5 (133)		141±54.8 (114)	
rec/10min	294±51.4 (108)		140±49.1 (79.2)		170±49.5 (133)		114±69.8 (91.7)	
ICA								
pre	319±28.1 (100)	P=0.0001	390±27.4 (100)	NS	340±27.8 (100)	NS	306±37.5 (100)	NS
post	385±23.4 (121)		455±34.7 (117)		353±29.6 (104)		382±48.8 (125)	
rec/10min	338±37.5 (106)		375±26.2 (96.1)		317±43.3 (93.1)		234±9.3 (76.4)	
MCA								
pre	214±22.4 (100)	P=0.01	235±21.5 (100)	NS	218±15.9 (100)	NS	216±14.7 (100)	NS
post	298±19.5 (139)		229±40.3 (97.4)		250±26.5 (115)		245±13.4 (113)	
rec/10min	201±26.1 (93.9)		223±40.8 (94.9)		210±15.3 (96.3)		158±5.8 (72.8)	
ACA								
pre	255±20.3 (100)	P=0.04	226±22.7 (100)	NS	226±18.6 (100)	NS	226±21.2 (100)	NS
post	282±32.4 (110)		265±24.2 (117)		230±20.8 (102)		274±19.4 (121)	
rec/10min	260±23.1 (102)		205±22.3 (87.2)		200±15.3 (88.5)		182±24.8 (80.5)	
PCA								
pre	180±28.5 (100)	P=0.01	251±15.0 (100)	p=0.007	220±46.6 (100)	NS	212±26.7 (100)	NS
post	226±19.7 (126)	P=0.007	313±18.4 (125)		207±41.0 (93.9)		258±18.8 (122)	
rec/10min	226±8.4 (145)		257±24.3 (102)		160±30.6 (72.7)		166±28.9 (78.1)	

Table 3. Comparative measurments in normal rats and SHR with subarachnoid hemorrhage

	WKY			SHR		
	SAH(-)	SAH(+)	p Value	SAH(-)	SAH(+)	p Value
Basilar A						
vessel wall(μm)	19.16±0.17	34.82±1.28	p=0.002	29.58±0.67	31.25±0.98	NS
vessel lumen(μm)	153.33±6.89	151.79±9.57	NS	110±7.29	83.33±5.00	NS
ICA						
vessel wall(μm)	29.16±0.83	29.29±1.08	NS	33.33±0.33	35.25±0.56	NS
vessel lumen(μm)	296.67±32.0	272.5±13.08	NS	217.5±10.79	197±14.26	NS

DISCUSSION

Angiographical Assessment of Vasospasm in Rodents

Measurements of rat cerebral arteries after subarachnoid hemorrhage have been reported using angiography (3, 8, 11, 17, 27), and observation with a cranial window (18, 22, 23). By mammographic equipment or selective biplane digital subtraction angiography systems, recent studies using rodents showed availability of measuring the vessel diameters with or without spasms (11, 26).

Magnification of the vessels using a mammography suggests the possibility of visualizing the small cerebral vessels (11, 12, 17). The small focus technique provides high geometric magnification with longer film - focus distance. This technique is especially useful in combination with digital subtraction techniques. They demonstrated the ability to view rat cerebral trunk arteries and some extracranial arteries including superior and inferior ophthalmic arteries, although the vessel diameters were not measured. The theoretical magnification of the mammography was suggested to be x 250 in the literature, however, one

concern with magnification increases, is radiation scattering which makes the margins of vessel image blurry.

Regarding the time resolution for rat cerebral angiography, some studies used anterograde injection of the contrast media by placing the cannula in CCA or ICA, thus the ipsilateral blood flow in ICA was disrupted (11, 12). With this preparation, the volumes of injected contrast media were between 0.2 to 0.3 ml. The obtained images showed cross filling of the contrast media to the whole contralateral hemisphere as well as the posterior fossa, suggesting that normal intracranial blood flow was absolutely changed at the time of imaging. Others used retrograde injection via the ECA to the CCA (17). An external carotid perfusion loop allows for the introduction of the contrast medium without changing perfusion pressure or flow in the cerebral hemisphere. However, the injection volume of the contrast media was as high as 0.5 ml and only a single angiography was performed in one experimental setting.

In the present study, we demonstrated the ability to obtain rat cerebral microangiograms using synchrotron radiation. Selective microangiography of hemispheric brain clearly showed not only the images of major trunk vessels but also of vessels less than 100 μm in diameter. The subtraction images were obtained every 0.33 sec and microangiography repeated up to five times in each rat, which is in contrast with previous studies where only two imagings could be done. This high quality of microangiography has not been reported by current conventional method of imaging. We believe that this can be obtained only by the combination of highly monochromatized SR and a new X-ray SATICON camera.

We found that the absolute value of vessel diameter in our studies was smaller than previous studies in the rat brain, in which angiography using mammography measured the diameter of the MCA in rat at 305-350 μm (11, 17). The diameter of the MCA in our study was at 201 +/- 20 μm in WKY. This diameter is close to that found in a histological study (15, 20) and we think, therefore, that our study is likely more accurate. Magnified images in mammography may have scattering radiation around the contrast media resulting in obscure margins and inaccurately larger diameter of the vessels. A large injection volume of the contrast media or proximal blocking of the ICA due to the catheter placement might cause the enlargement of the vessels that can not be detected by conventional methods used in previous studies.

Chronic Hypertension and Distensibility of Cerebral Arteries

In WKY, we observed vasospasm in basilar artery after SAH at 66% of pre-SAH diameter. The vasospasm was not observed in ICA, MCA, ACA and PCA. Actually, ICA and PCA showed a tendency of enlargement of the vessels after SAH, although which was not statistically significant (ICA: from 319 μm to 360 μm, PCA: from 180 μm to 251 μm). Interestingly, similar pattern of enlargement in ICA and PCA were observed in comparison between normotensive WKY and hypertensive SHR (ICA: 319 μm to 340 μm, PCA: 180 μm to 220 μm). We speculate that after SAH in WKY, ICA and PCA might be enlarged due to compensatory dilatation for severely affected spastic basilar artery that was mostly affected by SAH by blood injection into posterior cranial fossa. Similarly, in SHR, the basilar artery might be mostly affected because other major vessels have a collateral blood flow via the external carotid arteries.

We have demonstrated that the vasospasm was absent in basilar artery of the SHR. We suspect that the reason for absence of vasospasm was, at least in part, due to morphological change of vessel wall. In comparison of the thickness of basilar artery wall between WKY and SHR, SHR was significantly thicker than WKY (30 μm in SHR vs. 19 μm in WKY). Not only simply thick wall but also morphological changes of medial smooth muscle cells

and blood brain barrier have been reported in SHR (1, 15). Taken together, pre-existing affected distensibility in SHR might result in lack of spastic change after SAH. This may be also responsible for our observation of lack of distention by hypercapnia in SHR. The distensibility of affected vessels can be quantified only by high-resolution imaging. Regarding the impact of chronic hypertension on the distensibility of cerebral vessels, previous study using SHR and laser Doppler measurement of cerebral blood flow has been reported that pressure-dependent constriction of cerebral vessels was attenuated and lost after stroke (21). By hypercapnia, cerebral blood flow measurement by hydrogen clearance method resulted in no significant difference of the vasodilation between normotensive rats and hypertensive rats (6). Those previous studies were based on relative blood flow changes and direct measurement of the vessel diameter has never been performed. With large experimental animals, various studies have been reported but those were all non-pathological condition and no study could be done with chronic hypertension (4, 5, 7). Our study showed that major trunk vessels in SHR were less affected by SAH than in WKY, indicating that the degree of vessel damage is already maximized in SHR, leading high vulnerability. Similar result was obtained using young and aged rabbit with SAH (14).

Clinical implication of the present data

Our results in this study were mimicking of clinical observation in several points (10). Firstly, location of SAH clot is a key of induction of vasospasm, which is commonly observed in SAH patients and we found similar localized vasospasm in rodent. Secondly, the mortality of SHR after SAH was recognized high as two out of nine rats whereas zero in WKY. Pre-existing deteriorated autoregulation of the cerebral blood flow in SHR probably lead low blood perfusion in brain stem, resulting fetal ischemia under circumstances of high intracranial pressure with SAH. Thirdly, autoregulation of cerebral blood flow is affected even in normotensive WKY, indicating maintaining of systemic blood pressure is essential to prevent delayed ischemic damage after SAH. Impaired endothelium-dependent relaxation has been discussed recently as a main reason of such pathogenesis of distensibility (26). To evaluate such a mechanisms, not only a simple measurement of vessel diameters but also imaging studies like microangiography using vasodilator is useful.

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Short Communication

Dilation of perforating arteries in rat brain in response to systemic hypotension is more sensitive and pronounced than that of pial arterioles Simultaneous visualization of perforating and cortical vessels by in-vivo microangiography

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ABSTRACT

Autoregulatory responses of perforating arteries play a key role in the maintenance of microcirculation of the deep brain regions. The aim of this study was to test our hypothesis that autoregulatory vasodilatation of perforating arteries is more effective than that of cortical arteries. We performed cerebral microangiography in adult Wistar rats using monochromatic synchrotron radiation at SPring-8 and for the first time radiographically visualized perforating arteries and cortical arteries simultaneously in a single view. In response to hypotension induced by arterial bleeding, both arteries showed significant vasodilatation. Steady-state responses of increments in caliber to stepwise hypotension revealed that perforating arteries exhibited significant vasodilatation at blood pressure below 80–99 mm Hg. Cortical arteries, on the other hand, showed a gradual and smaller vasodilatation beginning at 60–79 mm Hg. For the lowest blood pressure range at 40–59 mm Hg, the smallest arteries with a diameter of 20–40 μm showed maximal dilation in both groups, but perforating arteries showed significantly larger dilatation (185.0% of baseline diameter) than cortical arteries (152.7%; $P=0.003$). Our results indicate that vasodilatation of perforating arteries is more sensitive and pronounced in response to systemic hypotension than that of pial arteries, which explains how cerebral microcirculation is maintained efficiently in the deep brain regions.

Introduction

Perforating arteries are terminal vessels located deep in the ventral brain area. They directly emerge from the main cerebral trunks without collateral flow with adjacent arteries. Perforating arteries are particularly important because they supply blood to vulnerable parts of brain structures, e.g., basal ganglia, thalamus and hippocampus. Pial arterioles are located on the cortical surface and are richly anastomosed, not only among themselves, but also with the branches from the external carotid artery (ECA). Such rich anastomosis can compensate for reduction in local perfusion during hypotension.

Autoregulation of cerebral blood flow (CBF), the tendency for blood flow to remain constant despite changes in arterial perfusion pressure, is a crucial mechanism for the maintenance of cerebral circulation. This autoregulation of blood flow is accomplished via constriction of cerebral blood vessels as the pressure rises and vasodilatation as the blood pressure declines (Kontos et al., 1978; Faraci and Heistad, 1998). Autoregulatory response of perforating arteries plays a key role in sustaining microcirculation of the deep brain regions and its disruption may be involved in the pathogenesis of small-vessel diseases such as lacunar infarction and leukoaraiosis (Jorgensen et al., 1994; Molina et al., 1999). However, so far it has not been possible to examine autoregulatory adjustments of perforating arteries radiographically because of their deep-seated anatomical location and small size. Nor has any comparative study been made of perforating arteries and pial vessels.

We recently developed a novel cerebral angiography procedure for rodents using monochromatic synchrotron radiation X-rays at SPring-8 and obtained images of cerebral perforating arteries (Morishita et al., 2006; Kidoguchi et al., 2006; Oizumi et al., 2006). In the study presented here, we improved this procedure to visualize rat perforating and cortical arteries simultaneously in a single view. We then used this method to test the hypothesis that, after induced hypotension, perforating arteries distend more efficiently than pial arteries to maintain constant blood supply to the deep brain regions.

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Material and methods

This study used nine 6-month-old male Wistar rats weighing 400–450 g. All of our experimental procedures were in accordance with the guidelines for animal experiments of Kobe University. The cerebral microangiography for rat brain used ultra-bright and monochromatic X-rays at the third-generation synchrotron radiation center SPring-8. Details of the microangiographic method have been described elsewhere (Umetani et al., 2007). Briefly, after anesthesia with pentobarbital sodium (50 mg/kg i.p.), bilateral femoral arteries were cannulated for the recording of mean arterial blood pressure and for taking arterial blood samples and inducing hemorrhagic stepwise hypotension. After the ECA had been cannulated to inject the contrast medium, the rats were placed in the supine position in a stereotaxic frame. Rats were intubated and the temperature monitored and controlled at 36–37 °C with a thermo-controlled heating pad. Artificial ventilation of air was maintained by a ventilator at a rate of 60–70 respirations/min. To visualize the perforating and cortical vessels of the hemisphere in a single-view 9.5 mm×9.5 mm frame, rats were tilted at 70° angles to the X-ray beamline and 7° angles in the vertical direction.

After preparation of the animals, the contrast tube inserted in the ECA was connected to an automated injector programmed to deliver 0.3 ml of non-ionic contrast media in 0.6 s. The first angiogram was then obtained to estimate the basal tone of the vessels and we allowed at least 3 min intervals between angiograms to reestablish physiological blood flow before the next angiographic study. The hypotension required to analyze the vascular reactivity of cerebral vessels was achieved by inducing arterial bleeding. The mean arterial blood pressure (MABP) of each animal was reduced in steps of 20 mm Hg at a rate of −0.22 mm Hg/s, and 4–5 successive angiograms were obtained. Before each angiogram, steady-state level of blood pressure was confirmed, which was defined as variance in blood pressure within 10% of MABP. At an MABP below 40 mm Hg, fragmentation of flow in the vessels under observation occurred frequently and made diameter measurement unreliable. We therefore avoided reducing the blood pressure to less than 40 mm Hg. Blood samples were obtained immediately after the first baseline angiography for the measurement of arterial gas tension, pH, and glucose concentration.

The images were stored digitally, and to generate the subtraction images, 10 original images were added to and subtracted from the pre-infusion image. Sequential images were obtained with an input field of 9.5 mm×9.5 mm view and pixel size of 9.5 μm per side. Quantitative measurement of vessel diameter changes by less than the pixel size was achieved by calculating the vessel diameters semi-automatically on the digital image with Image Pro Plus Ver. 5.0 (Media Cybernetics, Inc., Silver Spring, MD) combined with a program especially developed for this study (Oizumi et al., 2006). With this semi-automatic measurement, vessel intensity profiles orthogonal to a smoothed trace in the vessel are picked up, and the vessel diameter is estimated as the width of the intensity profiles.

Table 1
Baseline diameters of perforating and cortical arteries

Range of vascular diameter (μm)	Perforating arteries (μm)*	Cortical arteries (μm)*	P value
20–40	32.7±4.6	35.9±4.3	0.17
40–60	48.1±5.7	48.2±4.8	0.83
60–80	68.4±3.5	70.7±5.0	0.15
80–100	–	86.8±5.1	–
100–120	–	107.0±6.0	–
20–80	51.0±9.8	50.3±12.6	0.79

* Data were presented as average±SD.

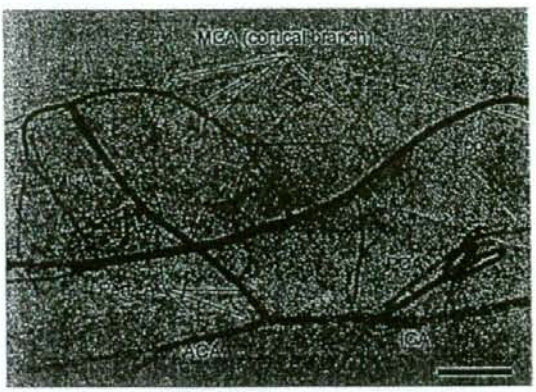

Fig. 1. A representative angiographic view of the cerebral vessels in hemisphere. ICA, internal carotid artery; ACA, anterior cerebral artery; MCA, middle cerebral artery; PCA, and posterior cerebral artery; Perf A, perforating arteries; ppA, pterygopalatine artery.

Statistical analysis

Data are shown as average±SD. Non-paired t-test and two-way analysis of variance (ANOVA) were used for comparisons among the groups. Post-hoc comparisons between mean values were made with the Dunnet test. P values <0.05 were accepted as statistically significant.

Results

Mean arterial blood pressure at baseline was 110.5±2.9 mm Hg, while arterial pH, PaO_2 and $PaCO_2$ were 7.41±0.03, 92±5 mm Hg, and 36±9 mm Hg, respectively. During hypotension, pH was 7.37±0.08, PaO_2 94±5 mm Hg, and $PaCO_2$ 34±5 mm Hg. Perforating arteries 20–80 μm in diameter and cortical vessels 20–120 μm in diameter were measured. We grouped cerebral vessels into five caliber sizes (20–40 μm, 40–60 μm, 60–80 μm, 80–100 μm, and 100–120 μm), because the changes in arterial caliber in response to changes in arterial blood pressure are size-dependent (Kontos et al., 1978). The mean vessel diameters of the groups are shown in Table 1, demonstrating that there were no significant differences in vascular caliber between perforating and cortical arteries.

A representative microangiographic view of the whole hemisphere is shown in Fig. 1. In this image we can clearly see the internal carotid artery, anterior cerebral artery, middle cerebral artery (MCA), posterior cerebral artery and perforating arteries. Some perforating arteries can be seen to emerge from the first portion of the MCA while the second and third ramification of the MCA could also be visualized in a single angiographic view.

Fig. 2A shows steady-state responses of caliber increment to stepwise hypotension of perforating and cortical arteries 20–80 μm in diameter. Baseline diameters of these arteries were not significantly different (Table 1), but perforating arteries exhibited significant vasodilatation at a blood pressure below 80–99 mm Hg and a progressive increase in vascular calibers in response to stepwise hypotension, while cortical arteries showed a gradual and smaller vasodilatation. Significant dilatation was observed in the cortical branches of MCA at a blood pressure below 60–79 mm Hg, so that the vascular response pattern of perforating and cortical arteries was also significantly different (ANOVA).

Because autoregulatory distensibility of cerebral vessels largely depends on the vascular size (Kontos et al., 1978), maximal vasodilatation at a blood pressure of 40–59 mmHg for each vessel size is shown in Fig. 2B. In response to induced hypotension, both perforating

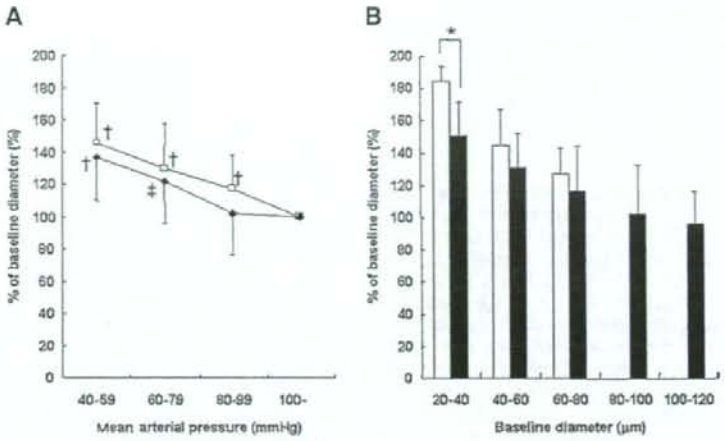

Fig. 2. (A) Steady-state responses of perforating and cortical arteries 20–80 μm in diameter to stepwise hypotension. Perforating arteries (white squares) exhibited significant vasodilatation at a blood pressure below 80–99 mm Hg and a progressive increase in vascular calibers in response to stepwise hypotension. Cortical vessels (black diamonds) showed a gradual and smaller vasodilatation. † and ‡ indicate $P<0.0001$ and $P<0.001$ vs. normal blood pressure, respectively (ANOVA). (B) Maximal vasodilatation of perforating and pial arteries in response to induced hypotension (40–59 mm Hg). Both perforating (white bar) and cortical (black bar) arteries showed significant dilatation. Perforating arteries 20–40 μm in caliber showed a 185.0% increase in dilatation, while cortical arteries of the same diameter showed a 152.7% increase compared with baseline diameter. Asterisks denote $P=0.003$ vs. perforating arteries.

and cortical arteries clearly showed vasodilatation and perforating arteries 20–40 μm in caliber showed 185.0% dilatation compared with the baseline diameter. Larger perforating vessels also dilated significantly, but the percentage increase in diameter in response to hypotension was greater in the smaller than in the larger vessels. On the other hand, cortical arteries showed less vascular dilatation than perforating arteries, with maximal dilatation of cortical vessels 20–40 μm in diameter of 152.7% ($P=0.003$ vs. perforating vessels).

Discussion

In this study, we modified the microangiographic technique by tilting the rat brain to the X-ray beamline so as to focus on autoregulatory response to hypotension of perforating vessels in comparison with that of cortical arteries. Our results clearly indicated that both perforating and cortical arteries dilated in response to induced hypotension and that regulation of distensibility of perforating vessels was more sensitive and pronounced than that of cortical arteries. To the best of our knowledge, this is the first in vivo evidence of a functional difference between perforating and pial arteries, which strongly supports the notion that effective autoregulatory responses of perforating arteries are crucial for maintaining microcirculation of deep brain structures.

Barzo et al. (1993) reported that the functioning of autoregulation in the deep gray matter is strongly influenced by the rate of change in systemic arterial blood pressure. They found that CBF remained at baseline values if hypotension was produced at a rate slower than −0.4 mm Hg/s. Otherwise, the reduction in CBF was similar to that in MABP. In our experiments, stepwise hypotension was induced slowly (−0.22 mm Hg/s) and the steady-state level of blood pressure was confirmed before each angiogram. Thus, it seems likely that autoregulatory responses of cerebral vessels were functioning at each step of hypotension induction.

Baumbach and Heistad (1985) pointed out the regional heterogeneity of cerebral vascular autoregulation, and Mueller, Heistad and Marcus (1977) showed that autoregulation of blood flow is more effective in the brain stem than in the cerebrum. In the pial arterioles, progressive vasodilatation has been demonstrated at a blood pressure below 80 mm Hg (Kontos et al., 1978), which agrees with our results. Our study also found that perforating arteries showed more pronounced and sensitive autoregulatory responses to changes in blood pressure. Three possibilities have to be considered to explain the regional differences in autoregulatory vascular responses: difference in the anatomical structure of vessels, difference in changes in intravascular pressure, and difference in regulation of autoregulatory vasodilatation.

Anatomical studies of human brain have revealed that pial arteries on the cortical surface are generally equipped with two to three muscle layers, whereas penetrating arteries have only one to two smooth muscle cells per circumference (Lamport and Baez, 1962; Edvinsson and Krause, 2001). The same structural elements are noted in rat cerebral vessels, but with smaller dimensions (Edvinsson and Krause, 2001). These structural differences of vessels suggest the wall of pial arteries is less elastic, while another possible implication is that the change of intravascular pressure in pial arteries is smaller. Autoregulatory changes in the caliber of the upstream larger vessels are sufficient to compensate for changes in arterial blood pressure and therefore pressure changes in pial arteries are smaller than the associated changes in mean arterial blood pressure (Lamport and Baez, 1962; Stromberg and Fox, 1972). In contrast, perforating arteries are more proximal and may more strongly reflect the changes in systemic blood pressure. Finally, mechanisms that mediate autoregulation of cerebral blood vessels may include myogenic responses, metabolic factors, neural mechanism and activation of potassium channels (Faraci and Heistad, 1998). Myogenic regulation, neural mechanism and metabolic influences surrounding perforating and cortical vessels may be different, but the precise mechanism of autoregulatory responses in perforating vessels remains unknown.

Because perforating arteries do not have any compensatory mechanism other than autoregulatory vasodilatation, it seems likely that the sensitive and pronounced response of these vessels is mainly responsible for sustaining microcirculation of the deep brain regions during hypotension, so that deterioration of this response could cause serious brain damage. Cerebral small-vessel disease is pathologically characterized by multiple lacunae and widespread white matter lesions, with hypertension as a major risk factor (Yanagihara, 2002, Khan et al., 2007). Stroke-prone spontaneously hypertensive rats (SHR-SP) provide an excellent model for small-vessel disease, because

these rats show chronic hypertension, structural alterations of small cerebral arteries, reduction in cerebral blood flow and white matter lesions (Yamori and Horie, 1977; Lin et al., 2001; Fujita et al., 2008). We recently also investigated autoregulatory responses of the perforating arteries in SHR-SP (Morishita et al., 2006). Our microangiographic studies revealed that perforating arteries of SHR-SP are already dilated under normal blood pressure and therefore lose their distensibility in response to induced hypotension. Pathological alterations of perforating arteries observed in SHR-SP may result in a reduced compensatory response to an increase in blood flow during hypotension. This implies that impaired vascular responses of perforating arteries deserve to be recognized as partly responsible for the pathogenesis of cerebral small-vessel disease.

Following limitations are considered. 1) Although changes of vascular caliber were consistently measured in this study, changes in local CBF during stepwise hypotension were not determined. Combined observation of CBF by means of laser-Doppler flowmetry (Barzo et al., 1993) would help to reveal the microcirculation of deep brain regions more precisely. 2) Angiographic analysis of the present study was performed under the anesthesia with pentobarbital sodium. Effects of barbiturates on the cerebral vessels have not been evaluated. However, any differential effects of pentobarbital on perforating and cortical arterioles have not been demonstrated (Hendrich et al., 2001).

On the other hand, angiographic imaging of the perforating arteries in comparison with cortical arterioles has several impacts for the medical community. This study facilitates the investigation of not only the microcirculation in the deep brain region, but also the pathogenesis of the cerebral small-vessel disease in atherosclerotic conditions, such as ageing, hypertension and diabetes. Further steps of research can be conducted to find the molecular basis of impaired cerebrovascular reactivity using the rodent models for neurological disorders (Kidoguchi et al., 2006).

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Factors associated with lower Mini Mental State Examination scores in elderly Japanese diabetes mellitus patients

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Abstract

Cognitive impairment in elderly diabetic patients has generated considerable interest recently; however, the mechanism of the impairment remains to be elucidated. In the current study, factors associated with cognitive dysfunction in old diabetic patients were explored. A Mini Mental State Examination (MMSE) was performed on 907 of 1173 registered elderly Japanese diabetic subjects. To characterize the clinical features of diabetes, we examined indices of glycemic control, lipid metabolism, blood pressure and complications. Single regression analysis adjusted for age showed that shorter height, higher GDS 15 scores, lower serum albumin, history of cerebrovascular disease, the existence of diabetic nephropathy, no smoking habit, no drinking habit, and no occupation were associated with lower MMSE scores. Multiple regression analysis demonstrated that age (odds ratio (OR) = 1.079; 95% confidence interval (CI) = 1.011–1.150), GDS 15 scores (OR = 1.139; 95% CI = 1.045–1.243), serum albumin (OR = 0.336; 95% CI = 0.174–0.745), and history of cerebrovascular disease (OR = 3.011; 95% CI = 1.578–5.748) were the variables significantly associated with having lower MMSE scores.

Keywords: Cognition; Dementia; Serum albumin; Cerebrovascular accident; Depression

1. Introduction

The prevalence and incidence of diabetes mellitus (DM) are increasing at all ages, including older populations, and approximately 15% of the elderly population in Japan is affected. Multiple metabolic abnormalities in DM induce systemic complications, which may include microangiopathic complications (neuropathy, retinopathy, nephropathy) and macroangiopathic atherosclerosis (stroke and ischemic heart disease). Several studies have shown that elderly diabetics have impaired cognition compared to age-matched non-diabetics, as well as a higher risk of dementia (Cukierman et al., 2005; Mogi et al., 2004; Strachen et al., 1997). Because the increase in the number of elderly people with cognitive impairment or dementia creates significant medical, social and economic burdens, cognitive impairment in older DM subjects has recently sparked considerable interest. It is highly desirable to be able to provide intervention in the case of older DM subjects who are at risk for cognitive decline or dementia in order to preserve cognitive functions; however, the mechanism of DM-associated cognitive decline remains to be elucidated and there is no solid evidence as yet that any treatment for DM is effective in preventing cognitive decline (Areosa Sastre and Grimley Evans, 2007). In order to establish an effective way of treating or preventing

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DM-related cognitive decline, the factors associated with this condition must first be determined. In the present study, we investigated factors associated with cognitive impairment in elderly DM subjects using baseline data from a large-scale cohort study of elderly DM in Japan.

2. Methods

2.1. Participants

The J-EDIT study was initiated in 2001 as a prospective intervention study of elderly Japanese people with DM for the purpose of determining how to prevent several diabetic complications. One thousand one hundred and seventy-three diabetic subjects were enrolled in 39 institutes and hospitals in Japan. They were all aged 65 years or more and had serum HbA1c levels at least 7.5%, or at least 7.0% with one of the following comorbidity factors: hypertension (130/85 mmHg and over), obesity (a body mass index (BMI) of at least 25), dyslipidemia (total cholesterol of at least 200 mg/dl, low-density lipoprotein (LDL) of at least 120 mg/dl, high-density lipoprotein (HDL) of 40 mg/dl or less, and/or triglyceride of at least 150 mg/dl). Although no exclusion criteria were determined for the registration of JEDIT, severely demented subjects were not selected because the filling out of several questionnaires was mandatory.

The study protocol was approved by the ethical committee in all of the enrolled institutes, and written informed consent was obtained from each patient.

2.2. Functional assessment

The Mini Mental State Examination (MMSE) was administered to most patients (907 of 1173) upon registration (Folstein et al., 1978). The MMSE is a global test of orientation, attention, calculation, language and recall with a score of 0–30.

Of the 1173 enrolled cases, MMSE scores were not collected in 266; data sheets were not returned in 48, subjects dropped out just after registration in 35, and doctors did not perform MMSE in 183.

Basic activities of daily living (BADL) was measured by a Barthel Index score of 0–20 (Mahoney and Barthel, 1965), and depressive mood was assessed by a short version of the Geriatric Depression Scale (GDS-15) (Yesavage, 1986).

2.3. Assessment of diabetes mellitus, complications and comorbidities

The diagnosis and patient data regarding DM, blood examinations and complications were obtained from clinical charts (The Expert Committee, 2003). After overnight fasting, blood samples were taken by venipuncture to assess serum levels of glucose, HbA1c, total cholesterol, triglyceride and HDL cholesterol. Additionally, serum insulin concentrations were determined in patients who were not receiving insulin therapy. Diabetic nephropathy was assessed according to the mean urinary albumin-to-creatinine ratio (ACR) and was classified as no nephropathy (ACR < 30 μg/mg) or existence of nephropathy (microalbuminemia: 30 ACR < 300 μg/mg or more advanced). Diabetic retinopathy was assessed by fundoscopic examination performed through dilated pupils by experienced ophthalmologists, and was classified into two categories: mild (no retinopathy or intraretinal hemorrhages and hard exudates), or serious (soft exudates, intraretinal microvascular abnormalities, venous calibre abnormalities, venous beading, neovascularization of the disc or other areas in the retina, preretinal fibrous tissue proliferation, preretinal or vitreous hemorrhage, and/or retinal detachment). Diabetic neuropathy was defined as either the loss of the Achilles tendon reflex without neuropathic symptoms including paresthesia, or the presence of neuropathic symptoms. Macrovascular complications were classified based on the presence or absence of coronary artery diseases, and/or a history of stroke. The existence of a current regular occupation and current habits of smoking, drinking and exercising were also assessed by questionnaire as yes (1) or no (0).

2.4. Statistical analysis

The subjects were divided into two groups, one with higher cognitive function, defined as having an MMSE score of 24 or more, and one with lower cognitive function, defined as having an MMSE score of 23 or less, according to the review by Tombaugh and McIntyre (1992). The groups were compared with respect to each factor by the Student's t-test for continuous variables or a χ^2-test for categorical variables. Logistic regression analysis including each factor as an explanatory variable was performed to search the association of the covariants and cognitive dysfunction indicated by an MMSE score below 24 after adjusting for age. Then, multiple logistic regression analysis was performed with the variables selected by this analysis and additional variables of interest. Spearman's rank correlation coefficient was calculated to confirm the relationship between serum albumin levels and MMSE scores.

3. Results

The background characteristics of the two MMSE score groups are shown in Tables 1 and 2. The average age was 74.0 years old in the lower MMSE-score group (23 and less) and 71.8 years old in the higher MMSE-score group (24 and more) (Table 1). The average HbA1c and FBG levels in the higher MMSE score group and lower MMSE score group were 8.0% versus 8.1% and 5.1 mmol/l versus 5.0 mmol/l, respectively (Table 1). At least about half of the participants had microangiopathic complications (nephropathy, retinopathy, or neuropathy) as shown in Table 2.

Table 1
Analysis by Student's *t*-test

Item	Higher	Lower	*p*-Value
Number	848	59	
Age (years)	71.8 ± 4.6	74.0 ± 5.1	<0.001
DM duration (years)	16.3 ± 9.7	17.1 ± 8.8	0.545
Height (cm)	155.8 ± 8.4	152.3 ± 8.6	0.002
Body weight (kg)	57.9 ± 10.2	57.7 ± 8.8	0.071
BMI	23.8 ± 3.5	23.9 ± 3.2	0.874
HbA1c (%)	8.0 ± 0.9	8.1 ± 1.1	0.766
FBG (mmol/l)	5.1 ± 0.3	5.0 ± 0.5	0.234
Systolic BP (mmHg)	135.4 ± 15.6	133.3 ± 19.3	0.391
Diastolic BP (mmHg)	74.9 ± 9.5	76.4 ± 11.2	0.288
LDL cholesterol (mg/dl)	120.9 ± 30.6	126.2 ± 35.7	0.201
HDL cholesterol (mg/dl)	56.4 ± 18.0	57.7 ± 18.4	0.567
Triglyceride (log)	4.7 ± 0.5	4.6 ± 0.5	0.353
Lp (a) (mg/dl)	23.1 ± 22.9	25.9 ± 23.5	0.362
Albumin (g/dl)	4.2 ± 0.4	4.1 ± 0.5	0.001
MMSE	28.5 ± 1.8	20.3 ± 3.0	<0.001
ADL	19.9 ± 3.4	18.9 ± 1.0	<0.001
GDS-15	4.0 ± 3.1	5.9 ± 3.9	<0.001

Higher: the group with higher MMSE scores (24 or more), Lower: the group with lower MMSE scores (23 or less).

Analysis by Student's *t*-test showed that age, height, activities of daily living (ADL) scores, and serum albumin were significantly different between the two groups of patients (Table 1). A history of cerebrovascular disease, existence of diabetic nephropathy, current smoking habit, current drinking habit, and absence of occupation were also demonstrated to have a significantly different distribution between the two groups (Table 2). Fasting serum insulin levels or insulin treatment were not significantly associated with MMSE scores.

To determine variables significantly associated with cognitive dysfunction, logistic regression analysis adjusted for age was performed. The variables selected by this analysis were age, body height, serum albumin, the existence of an occupation, smoking habits, drinking habits, the existence of nephropathy, GDS-15 scores and history of cerebrovascular disease (Table 3). Then, multiple regression analysis was performed with all these significant variables plus variables of interest (HbA1c and gender) considered simultaneously. As shown in Table 4, higher age, higher GDS-15 scores, lower serum albumin and a history of cerebrovascular disease were significantly associated with the group having lower MMSE scores.

Table 2
Analysis by χ^2-test

	Higher	Lower	*p*-Value
Male	45.9 (389)	40.0 (23)	0.304
Existence of current occupation	67.2 (552)	47.4 (27)	0.002
Existence of exercise habit	61.1 (497)	48.3 (28)	0.055
Current drinking habit	40.4 (343)	25.4 (15)	0.017
Current smoking habit	46.5 (383)	31.0 (18)	0.022
Existence of nephropathy	48.5 (411)	64.4 (38)	0.018
Existence of retinopathy	48.8 (413)	60.8 (35)	0.088
Existence of neuropathy	65.5 (544)	73.2 (41)	0.241
User of antihypertensive drugs	55.2 (468)	62.7 (37)	0.261
User of antidyslipidemic drugs	38.8 (329)	42.4 (25)	0.586
Antiplatlet user	26.9 (227)	49.2 (29)	<0.001
Presence of IHD	17.6 (149)	16.3 (9)	0.650
History of cerebrovascular disease	12.6 (107)	32.2 (19)	<0.001

Higher: the group with higher MMSE scores (24 or more). Lower: the group with lower MMSE scores (23 or less). Data are expressed as percentages of the total with the number in parentheses.

Table 3
Univariate regression analysis adjusted with age

	Odds ratio	95% CI	*p*-Value
Height (cm)	0.959	0.928–0.992	0.015
Gender (male)	1.232	0.714–2.126	0.453
HbA1c (%)	1.033	0.779–1.369	0.822
Systolic blood pressure (mmHg)	1.005	0.988–1.021	0.576
Diastolic blood pressure (mmHg)	1.017	0.990–1.044	0.230
Albumin (g/dl)	0.322	0.163–0.637	0.001
History of cerebrovascular disease	3.128	1.735–5.637	<0.001
Existence of nephropathy	1.877	1.079–3.264	0.026
Existence of retinopathy	1.730	0.998–2.997	0.051
Existence of neuropathy	1.369	0.742–2.527	0.315
Existence of current occupation	0.498	0.287–0.863	0.013
Current drinking habit	0.527	0.287–0.968	0.039
Current smoking habit	0.544	0.305–0.968	0.038
GDS-15	1.166	1.080–1.259	<0.001
ADL	1.019	0.998–1.042	0.0810

95% CI: 95% confidence interval.

Table 4
Multiple logistic regression analysis

	Odds ratio	95% CI	*p*-Value
Age (years)	1.079	1.011–1.150	0.021
Height (cm)	0.954	0.905–1.006	0.083
Gender (male)	0.429	0.139–1.323	0.141
Albumin (g/dl)	0.336	0.174–0.745	0.006
HbA1c (%)	0.965	0.703–1.325	0.828
History of cerebrovascular disease	3.011	1.578–5.748	<0.001
Existence of nephropathy	1.679	0.913–3.089	0.096
Existence of current occupation	0.725	0.348–1.368	0.321
Current smoking habit	0.516	0.223–1.195	0.123
Current drinking habit	0.601	0.274–1.315	0.202
GDS-15 scores	1.139	1.045–1.243	0.003

95% CI: 95% confidence interval.

MMSE scores and serum albumin levels were significantly correlated based on Spearman's correlation (coefficient = 0.14902, $p < 0.001$).

4. Discussion

The analysis of the data from the J-EDIT study at registration demonstrated that a history of cerebrovascular disease, a low serum albumin level, higher GDS scores, and higher age were independently associated with lower cognitive function.

The present study demonstrated that in DM subjects, the strongest risk factor for cognitive dysfunction as defined by a MMSE score less than 24, which is considered to be the level defining dementia (Tombaugh and McIntyre, 1992), was a

history of stroke. Although the causes of cognitive dysfunction were not determined in the present study, vascular lesions might play a prominent role in the cognitive decline of DM subjects with a history of stroke. Furthermore, Snowdon et al. report that among subjects who met the neuropathological criteria for Alzheimer's disease, those with brain infarcts had poorer cognitive functions and a higher prevalence of dementia (Snowdon et al., 1997); thus, cerebrovascular disease might shorten the period of preclinical dementia. In the current study the participants were all Japanese, a race which is relatively prone to cerebrovascular diseases (Kitamura et al., 2006). The prevalence of a history of stroke in the current study was 13.9% (126 out of 907 participants), much higher than the 1.8% reported by Kuusisto et al. (1994) in Finland, and comparable to 18.8% in PROACTIVE, a secondary prevention study for macrovascular disease in diabetic patients performed in European countries (Charbonnel et al., 2004). Thus, the higher stroke prevalence might have affected the results of the current study.

Lower levels of serum albumin, even within the "normal" range, are associated with increased risks of stroke and coronary heart disease incidents as well as all-cause and cardiovascular mortality (Shaper et al., 2004). Of particular interest are several lines of evidence demonstrating that chronic inflammation is involved in atherosclerotic mechanisms, and high-serum proinflammatory factors including c-reactive protein, interleukin-6 and tumor necrosis factor have been reported to be risk factors for progressed atherosclerosis; these proinflammatory factors reportedly suppress the synthesis of albumin in the liver (Chojkier, 2005). The present results indicate that lower serum albumin and a history of cerebrovascular disease are independent factors associated with cognitive decline. However, asymptomatic strokes may also be involved in the mechanism of cognitive impairment in elderly diabetic patients (Araki and Ito, 2002). Although lower serum albumin was strongly associated with cognitive decline, mean urinary ACR was not associated with MMSE scores (data not shown).

The scores of GDS-15, which assessed depressive mood, were significantly associated with lower MMSE scores. The association of a depressive mood with cognitive dysfunction has been reported (Jorm, 2000). However, the mechanism of this association remains to be elucidated (Jorm, 2000). Cognitive dysfunction and depression may share common risk factors, depression may be a risk factor or prodrome of cognitive dysfunction, depression may affect the threshold of cognitive dysfunction, or depression may be a causal factor in cognitive dysfunction. Further analysis of longitudinal data of JEDIT study may shed light on this subject.

Many population-based and clinical studies have shown that DM is associated with cognitive decline in the elderly (Cukierman et al., 2005; Mogi et al., 2004; Strachen et al., 1997). Several hypothetical mechanisms have been suggested for this impairment; however, their clinical relevance is unclear (Biessels et al., 2006). The J-EDIT study was an interventional prospective study with a randomized control design. Longitudinal clinical and cognitive assessment of elderly diabetic patients will provide more information on the mechanisms of DM-related cognitive disorders.

Some limitations should be considered in the present case. First, the present study was performed with cross-sectional design using the data obtained at registration for the J-EDIT study. The patients are being followed longitudinally, and a follow-up analysis will be reported in the future. Second, because all of the patients enrolled were diabetic, it was not clear whether or not the results of the present study were diabetes-specific. In particular, the involvement of low serum albumin in the mechanism of cognitive decline in non-diabetic elderly patients should be investigated. Third, the present study did not include brain imaging. A subgroup analysis of J-EDIT subjects who underwent brain magnetic resonance imaging (MRI) was recently reported elsewhere (Akisaki et al., 2006), and revealed that cognitive decline in diabetes was associated with white-matter hyperintensities and subcortical atrophy in the tested subgroup. However, the relationship between the present results and the results of MRI analysis requires further investigation.

In the present study, neither DM-specific clinical indices including HbA1c, fasting blood glucose and serum insulin level, nor DM-related microangiopathies (nephropathy, neuropathy, and retinopathy) were associated with lower MMSE scores. The J-EDIT study recruited patients with relatively severe DM status, and this group of patients therefore did not represent the general population of elderly diabetics. The criteria for diagnosis for microangiopathy in the present study were relatively simple. Retinopathy has been reported as being associated with cognitive impairment or brain atrophy (Musen et al., 2006; Wong et al., 2002); proteinuria, which is a symptom of diabetic nephropathy, has received attention as a risk for stroke and ischemic heart disease (Madison et al., 2006); dysfunctions of the central and peripheral nervous systems may share a common pathogenesis (Gispen and Biessel, 2000; Suzuki et al., 2006). Further investigation of subjects with a broader clinical background and more sensitive diagnostic criteria for DM-related microangiopathic complications is required.

In conclusion, based on the results obtained in the current cross-sectional assessment, the prevention of cerebrovascular disease may be a primary way of preventing cognitive decline in elderly DM subjects. An investigation of how lower serum albumin levels are associated with DM-related cognitive impairment may lead to the development of effective strategies for the prevention or treatment of this decline.

Conflict of interest declaration

There is no conflict of interest for any of the authors.

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